THE OHIO JOURNAL OF SCIENCE

VOL. XLV NOVEMBER, 1945 No. 6

STUDIES IN THE BIOLOGY OF THE LEECH IX[1]

THE GROSS NERVOUS SYSTEM

JOHN A. MILLER

Department of Zoology and Entomology
The Ohio State University, Columbus, Ohio

INTRODUCTION

To the student of animal behavior a knowledge of the nervous system of the organism under observation is of utmost importance. Experimental evidence has provided valuable data regarding the arrangement of the functional components of the action system of this organism but in a comprehensive analysis of behavior this source of information is inadequate. A review of the work of earlier investigators is informative and applicable in so far as they have revealed the anatomical organization of the nervous system of annelids in general. The work of Whitman, Bristol, and others is especially pertinent at this point inasmuch as they were concerned with *Hirudinea*, and in the case of the latter, with a form closely related to the species herein described. I shall undertake in this and subsequent papers to present the macroscopic and microscopic anatomy of the leech *Haemopis marmoratis* (Say).

I have omitted from the following discussion any details of a cytological and histological nature except where imperative. This phase of the study is now under careful consideration and will appear at an early date. The writer has attempted to avoid a lengthy description of details in instances where a suitable illustration can best serve.

METHODS

In previous papers (1943–44) the writer has outlined in detail new staining methods and modifications applicable to the leech. In general it may be repeated here that the gold chloride and silver nitrate impregnation methods provide the basis for subsequent nerve differentiation. Counterstaining with phosphotungstic acid and Masson's stain has advantages in certain instances where tissue differentiation is also desirable. All of the illustrations, except where otherwise noted, are tracings of projected sections. Reconstructions are produced from serial sections employing micro-projection equipment.

THE NERVOUS SYSTEM OF HAEMOPIS MARMORATIS

The gross nervous system of *Haemopis marmoratis* is for convenience described as being composed of the following four divisions. (1) The central system, (2) the peripheral system, (3) the subepidermal system, and (4) the visceral system.

THE CENTRAL NERVOUS SYSTEM

The central system consists of an anterior cephalization contained in the head region, a series of segmentally arranged ganglia connected by the ventral nerve

[1] *Haemopis marmoratis* (Say).

cord in the body region, and a caudalization found in the anal region. The anterior division of this system is composed of a mass of neuromeres more or less completely fused, and forming a collar about the esophagus. The dorsal mass is referred to as the brain or supra-esophageal ganglion. This portion is connected with the ventrally located sub-esophageal ganglia by the circum-esophageal connectives (Plate No. II, Fig. 1). This anterior cephalization represents six neuromeres. The criteria used in this determination conforms with that employed by Whitman (1895) and Bristol (1899) in similar studies involving the metamerism of *Clepsine* and *Nephelis*, respectively.

The anal or caudal ganglia is composed of eight neuromeres. In this region fusion and distortion has taken place but not to the same degree as in the head region just described. Here the number and arrangement of the contributing neuromeres can be easily distinguished. A reconstruction from serial sections, using the same criteria as before, unmistakably established the boundaries of each ganglion. The number and position of lateral nerves arising from this caudal mass corresponds with the capsular number and arrangement. There are eight pairs of lateral nerves, one pair emanating from each determined ganglion.

Lying between the above described terminal portions are twenty ganglia. The first eighteen are metamerically arranged, though at the extremities not equally spaced. The nineteenth and twentieth ganglia are contained within somite XXV. The first three and the last two ganglia in the ventral chain are smaller than the the ganglia of the mid-body region. The ganglia of the ventral chain are joined together by a pair of nerve trunks extending from the sub-esophageal ganglion to the anal ganglia (Plate No. I, Fig. 1). The entire ganglionic chain is contained within the ventral blood sinus.

A Typical Neuromere

The knowledge of the capsular arrangement of the nerve cell bodies in a typical ganglion is a prerequisite to the analysis of either the "brain" or "anal ganglia." The nerve cell bodies of a ganglion are arranged, in general, in six primary clusters. Two such groups occupy a median ventral position while the remaining four are dorso-laterally placed. Near the center of the ganglion the cells of the latter are found to extend to a ventral-lateral position. It is only in the brain and anal ganglia that a shift from this general arrangement is noted. The number of primary capsules, however, even at the extremities remains constant. Each primary capsule is composed of numerous secondary cell clusters.

Of equal importance in defining the limits of a neuromere are the extra capsular cell bodies. Metamerically arranged cell bodies of this type may be listed under one of two classes. First, those whose cell bodies lie within the plexiform substance and whose processes enter the capsular area. The "Gross" motor nerve cell previously described by Krawany (1905), Retzius (1892), Smallwood (1930) and others is an example of this type. The cell body of the "Gross" cell is outside the capsule and usually contained within the plexiform or fibrous portion of the ganglion. These are giant multipolar motor cells. (Illustrated Plate I, Fig. 1, Miller 1944).

In *Haemopis*, as in *Nephelis* (Bristol) and *Clepsine* (Whitman) each body neuromere contains two "median nerve cells." The cell body of this type of "giant" cell is spindle shaped. (Plates Nos. II and III, Figs. 3 and 5). The cell bodies lie within the plexiform or fibrous portion of the ganglion in a ventral-median position. The number and arrangement of the "median nerve cell bodies" is especially valuable as an aid in determining the limits of the neuromeres contributing to the "anal ganglia."

A second class of extra-capsular nerve cells are those whose processes do not enter the capsular area. Two examples of this type of cell are found in the central system, both are "giant" bipolar cells.

The first example, Leydig's cell (Herman 1875) in addition to being outside the capsular aggregation has no connection with the plexiform or fibrous portion of the cord. It is present throughout the ganglionic chain, varying in position from between the axis of the lateral nerves of a midbody ganglion to an extra ganglionic position in the head and anal region. In the midbody region the processes of this cell leave the ganglion by way of the lateral nerves. The metameric consistency of Leydig's cell makes it a useful landmark in those areas where congestion of neuromeres has occurred. (Plate No. III, Fig. 1).

A second example of "giant" cell belonging to class two is the "colossal axial cell." (Plate No. III, Fig. 4). This type of cell will be mentioned only briefly inasmuch as it does not afford a basis for determining the limits of a neuromere. The nucleus of this cell lies about midway between the ventral ganglia within each of the two ventral connectives. The processes extend both anteriorly and posteriorly in the fibrous portion of the ventral cord. There is an experimental basis for inferring that the processes of the axial cell extend beyond the adjacent ganglia. Although my preparations clearly show the presence of inter-ganglionic processes continuing through the ganglion, a single fiber cannot be traced with accuracy over so great a distance.

I have excluded from the discussion at this time the extraganglionic neurons of the visceral and subepidermal systems. Reference to these systems will be made later in the paper.

Each body segment in *Haemopis marmoratis* is represented in the nervous system by a ganglion. The number of segments is established as XXXIV. The distribution of the ganglia and segments is illustrated in Table No. 1. The ganglionic centers of the nervous system are simple repetitions, element for element, the "brain" and "anal ganglia" not excepted. Plate Nos. 1 and II, Figures 1, 2, 3, illustrate the numbers and arrangement of the ganglia together with their lateral nerves for the head, body and caudal regions.

The Head Region

The head region is designated as that portion of the organism which includes the entire anterior cephalization. In *Haemopis marmoratis* this region extends from somite I through somite VI. This region includes the first eleven annuli.

The supra-esophageal ganglion or brain rests upon the dorsal wall of the pharynx at the level of the caudal annulus of somite V. Beneath the pharynx at the level of the second annulus of somite VI lie the sub-esophageal ganglia. The esophageal connectives extend laterally and ventrally from the brain. They are directed caudad, traversing the first annulus of somite VI, and join the sub-esophageal ganglia beneath the pharynx.

In order to determine the number of neuromeres comprising this region it is essential that the capsular number and arrangement as well as the peripheral nerves emanating from it be established. Nerve trunks one and two arise together from the anterior surface of the lateral connectives. They arise as parallel nerves proceeding anteriorly some distance before separating. The nerve cell bodies comprising the capsules of the first neuromere are located on the dorsal surface of the brain. The capsules of nerve two are located on the connectives. The third and fourth pair of lateral nerves arise near the vortex of the sub-esophageal ganglia. Both pairs divide into a dorsal and ventral branch some distance anteriorly. The fifth pair of nerves arises near the center of the sub-esophageal ganglia. These nerves proceed anteriorly to the level of the connectives as a single trunk before dividing into a dorsal and ventral nerve root. The sixth and last pair of nerves emanating from the head region, arise from the apical end of the sub-esophageal ganglia. Members of this pair divide soon after leaving the body of the ganglia (Plates Nos. I, II, Fig. 1).

The number of neuromeres is thus determined to be six. Whitman (1895) established the same number and essentially the same position for the neuromeres

in *Clepsine*. Bristol (1899) found a like number and position to prevail in *Nephelis lateralis*.

The Body Region

A complete body segment or somite consists of five annuli. Associated with each somite is a single ganglion located within the ventral blood sinus. In *Haemopis marmoratis*, eighteen ganglia are contained within the body region. The limits of this region are herein defined as somites VII through XXIV.

Extending caudad from the sub-esophageal ganglia are two parallel nerve trunks. Each nerve trunk is surrounded by a connective tissue sheath. The two main trunks are contained within an envelope which is continuous throughout the central nervous system. It has been previously stated that the ventral nerve cord, as well as the segmentally arranged ganglia of the ventral chain are contained within the ventral blood sinus. The first ganglion (most cephalad) of the ventral chain is contained within the first annulus of somite VII. This is the first in a series of segmentally arranged ganglia of the body region. It is actually the seventh ganglion of the animal and although smaller in size than a mid-body ganglion it is complete in every respect. From each ganglion of the body region arise two pairs of lateral nerves. The ganglia of the ventral chain, exclusive of the caudal region, occupy the first annulus of each somite.

The Anal Region

The anal region includes somites XXV through XXXIV inclusive. Somite XXV consists of two annuli, annulus 102 and 103 respectively. Somite XXVI includes annuli 104 and 105. Somite XXVII is represented by a single annulus, 106. Somites XXVIII through XXXIV are contained in the sucking disc.

Ganglion XIX of the ventral chain (the twenty-fifth of the entire series) is located within annulus 102. The XXth ganglion is contained in annulus 103. The last two mentioned ganglia are smaller than typical midbody ganglia. Each, however, contains six primary capsular aggregations of nerve cell bodies. Ganglion XX differs from the other unfused ganglia of the ventral chain in that it gives rise to a single pair of lateral nerves in deference to the usual anterior and posterior pair.

The "anal ganglia" is composed of eight neuromeres, and occupies the posterior third of annulus 103, all of annulus 104 and the anterior part of annulus 105. The components of this compressed and fused ganglia retain their fundamental characteristics and are plainly resolvable. The exact number and limits of each neuromere is established through reconstruction of the region and presented in Plate No. 11, Fig. 3.

In the preceding pages I have stated that the adult *Haemopis marmoratis* is divisible into thirty-four segments. This number is characteristic for *Hirudinea* in general as the work of many previous investigators will testify. The results of this study confirm earlier work in this respect, but I submit in deference to some that the allocation of specific segments to a particular body region cannot be made by generalizing from a study of a species or two.

EXPLANATION OF PLATE I

FIG. 1. This is a dorsal view of the anterior portion of *Haemopis* illustrating the metameric position of the central and peripheral nervous systems within the first seven somites. The somites are indicated by Roman numerals and the annuli are numbered in Arabic figures. The paired peripheral nerves are illustrated on the right and are numbered by Roman numerals. (The nerves on the left side are omitted for clarity).

ABBREVIATIONS

sub. eso. g.....subesophageal ganglia supra. eso. g.....supraesophageal ganglia
v.n.c.....ventral nerve cord

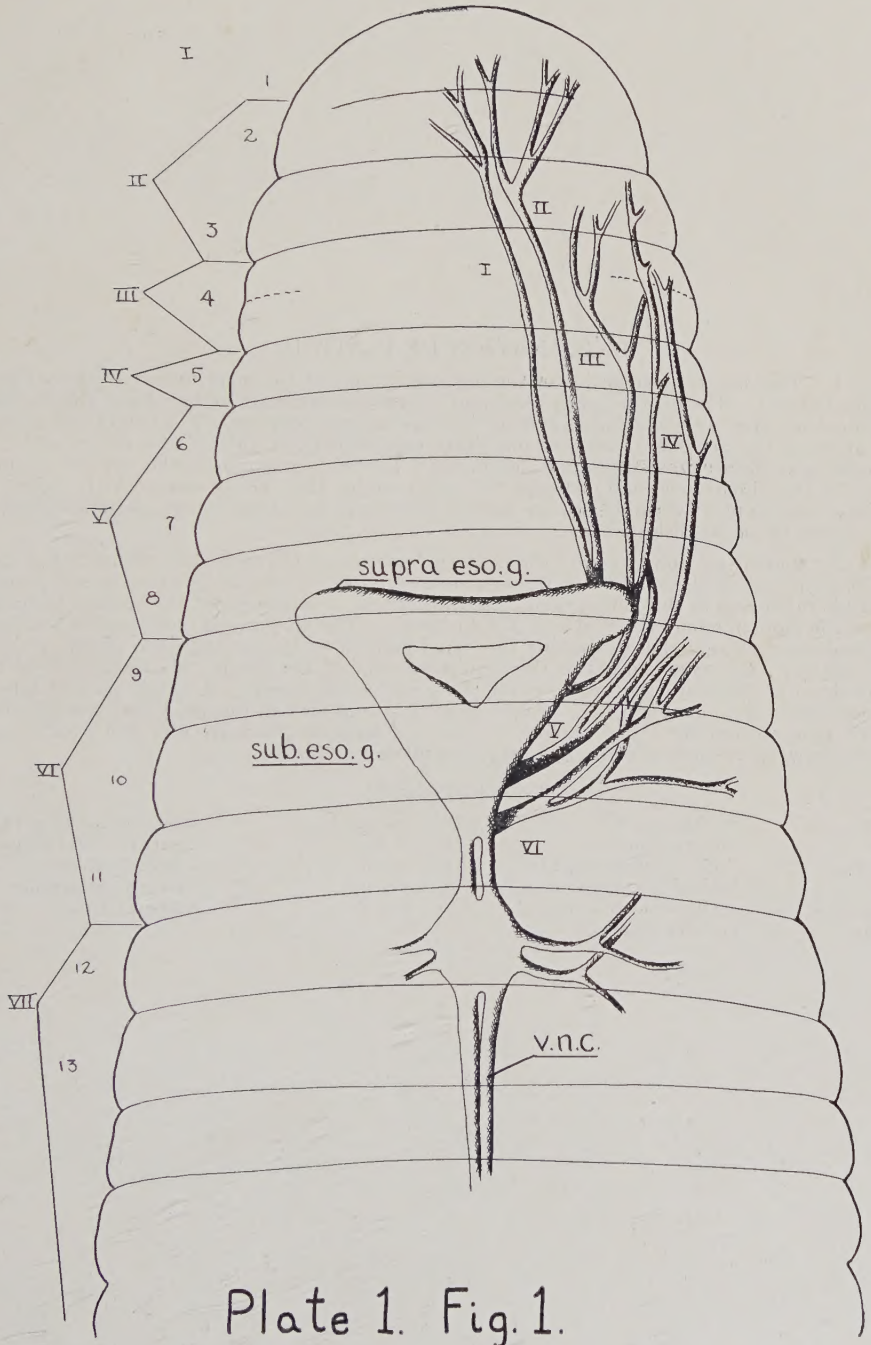

I
1
2
II
3
III
4
IV
5
6
V
7
8
9
VI
10
11
12
VII
13
I
II
III
IV
V
VI
supra eso. g.
sub. eso. g.
v.n.c.

Plate 1. Fig. 1.

EXPLANATION OF PLATE II

FIG. 1. This is a reconstruction of the nervous system of the head region as viewed from the lateral aspect. The central, peripheral and visceral systems are included in a single drawing to illustrate their relationship as well as their metameric position. The origin and general distribution of the peripheral nerves emanating from the right side of the collar and subesophageal ganglia are illustrated and numbered. The first ganglion of the ventral chain is included in this illustration and occupies the first annulus (No. 12) of somite VII. The visceral nervous system, as viewed from the right side, shows a ganglionic mass, circumesophageal trunks and anterior-posterior fibers.

FIG. 2. This is a lateral view of the posterior portion of *Haemopis* as reconstructed from serial longitudinal sections. The anatomical relation and metameric position of the ganglia and peripheral nerves in the caudal region are illustrated. The ganglia of the ventral chain are numbered in Roman numerals from I to XX inclusive. The six ganglia contained in the head region and the eight ganglia comprising the "anal ganglia" together with the twenty ganglia of the ventral chain constitute the thirty-four ganglia of the leech. (Ganglia XX of the ventral chain is therefore the twenty-sixth ganglion of the leech.) A single pair of lateral nerves emanates from ganglion XX. Eight pairs of lateral nerves, one from each contributing ganglion, extend from the "anal ganglia." The five anterior pairs are directed caudad, the posterior three pairs unite in forming a large caudal nerve trunk.

ABBREVIATIONS

anal. g. . . . . anal ganglion
ant. n. . . . . anterior nerve
d. l. cap. . . . . dorso-lateral capsule
l. n. s. . . . . lateral nerves
med. n. c. . . . . median nerve cell body
post. n. . . . . posterior nerve
sub. eso. g. . . . . subesophageal ganglia
v. b. s. . . . . ventral blood sinus
vis. f. . . . . visceral fibers
vis. g. . . . . visceral ganglion
vis. t. . . . . visceral trunks

Fig. 1.

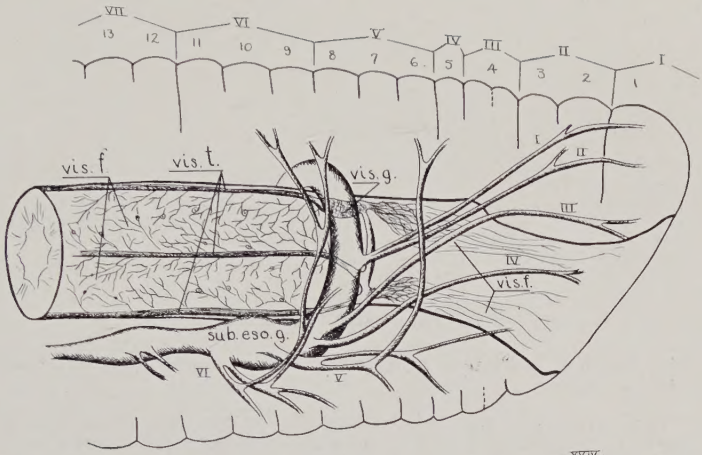


Fig. 2.

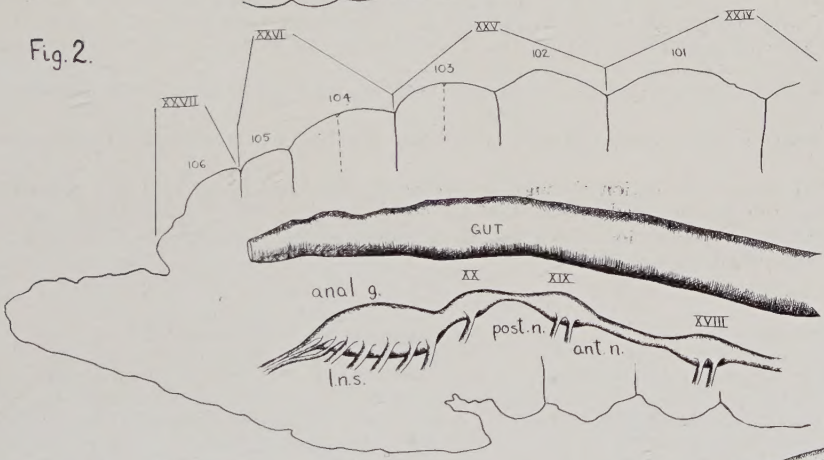


Fig. 3.

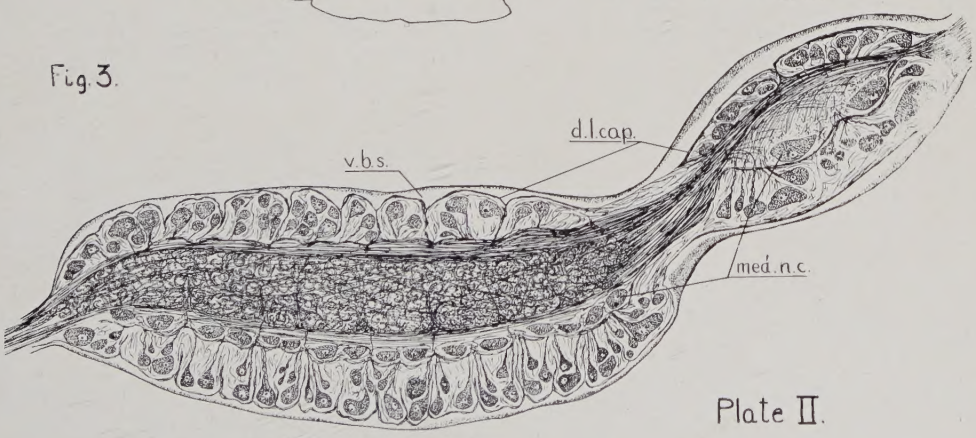


Plate II.

EXPLANATION OF PLATE III

Note.—The drawings in Plate III are traced from projected sections. Each with a magnification of 50 diameters.

FIG. 1. This is a horizontal longitudinal section through the dorsal hemisphere of a mid-body ganglion. A portion of each of the dorsal paired capsular areas (d. p. cap.) is illustrated. Many fiber tracts, nerve cell bodies and processes have been omitted in order to illustrate the general pattern. Leydig's cell (L. c.) is shown in the extra-capsular area between the anterior and posterior lateral nerves.

FIG. 2. This is a cross-section through a mid-body ganglion at the level of the posterior nerve.

FIG. 3. This is a cross-section through a mid-body ganglion just anterior to the anterior nerve.

FIG. 4. This is a cross-section through the ventral nerve cord about mid-way between ganglia. This section was selected to illustrate the cell body of a colossal axial cell. (c. a. c.)

FIG. 5. This is a cross-section of a mid-body ganglion at the level of the cell body of a median nerve cell. (med. n. c.)

ABBREVIATIONS

ant.	anterior
ant. l. n.	anterior lateral nerve
a. p. t.	anterior-posterior fiber tracts
c. a. c.	colossal axial cell
d. l. f.	dorsal longitudinal fiber tracts
d. p. cap.	dorsal primary capsule
ex. cap.	external capsule
int. cap.	internal capsule
L. c.	Leydig's cell
l. f.	longitudinal fibers
l. n.	lateral nerve
med. v. cap.	median ventral capsule
med. n. c.	median nerve cell
plex. sub.	plexiform substance
post.	posterior
post. l. n.	posterior lateral nerve
v. b. s.	ventral blood sinus
v. l. b.	ventral longitudinal fibers

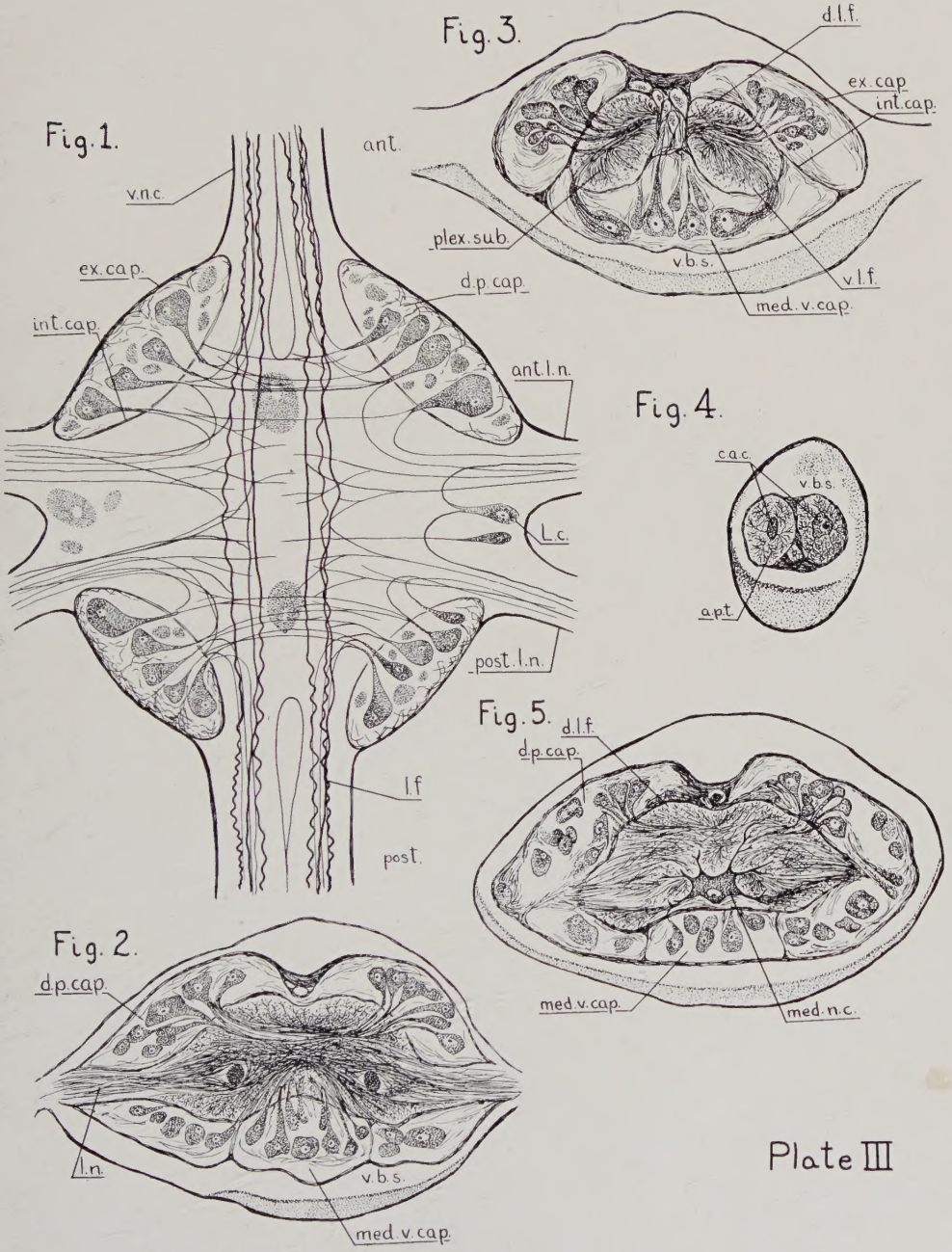


Fig. 1.
v.n.c.
ex.cap.
int.cap.
ant.
ant.l.n.
L.c.
post.l.n.
l.f
post.
Fig. 3.
d.l.f.
ex.cap
int.cap.
plex.sub.
d.p.cap.
v.b.s.
v.l.f.
med.v.cap.
Fig. 4.
c.a.c.
v.b.s.
a.p.t.
Fig. 5.
d.l.f.
d.p.cap.
med.v.cap.
med.n.c.
Fig. 2.
d.p.cap.
l.n.
v.b.s.
med.v.cap.
Plate III

Digitized by the Internet Archive
in 2025

https://archive.org/details/ohio-journal-of-science_1945-11_45_6

I have defined three regional divisions for the leech under consideration, namely, the head region, the body region and the anal region. In each division the number of neuromeres correspond to the number of segments, as follows, the head region six; the body region eighteen; and the anal region ten. I classify however, the first two ganglia (XXV and XXVI) of the anal region as ganglia of the ventral chain exclusive of the "anal ganglia." Thirty-four ganglia are thus determined and distributed, one for each body segment.

TABLE I

Head Region consists of:	Head Region contains:
Somites I to VI incl.	Ganglia I to VI incl.
Body Region consists of:	Body Region contains:
Somites VII to XXIV incl.	Ganglia VII to XXIV incl.
Anal Region consists of:	Anal Region contains:
Somite XXV	Ganglia XXV and XXVI
Somite XXVI	Ganglia XXVII to XXXIV
Somites XXVII to XXXIV	No ganglia

THE PERIPHERAL NERVOUS SYSTEM

In this system are included the paired lateral nerves and their branches emanating from the ganglionic masses of the central nervous system. From each ganglion of the body region there arises two pairs of lateral nerves. They emerge from the lateral border of the ganglia extending in a horizontal plane at right-angles to the long axis of the body. The anterior pair emerges near the center of the ganglion, the second pair just posteriorly to the first. Each lateral nerve separates a short distance from the ganglion into a dorsal and ventral branch. The divisions and subdivisions of these lateral nerves innervate a portion of the preceding segment as well as the segment in which they arise.

The nerves of the anterior pair are not single nerves as careful study will disclose. Each one is formed by the fusion of a dorsal and ventral root. The fibers of the dorsal and ventral root combine to form a single nerve trunk at the point where the lateral (anterior) nerve leaves the body of the ganglion. There is no evidence that the posterior pair arises in the manner just described for the anterior pair. It might be of interest to state that in certain leeches, *Clepsine* for example, there are three pairs of lateral nerves per segment. It has been suggested by some writers that in those leeches possessing but two pairs of lateral nerves per segment, that the anterior pair represents a fusion of the first two in those forms having three pairs per segment.

Exceptions to the above described pattern in the midbody region are found in the head and anal regions. In the head region a single pair of lateral nerves emanates from each of the first five established ganglia. The branches of these five extend to the anterior margin of the prostomium and oral sucker. From the sixth and last ganglion of the head region emerge two pairs of lateral nerves. These innervate the sixth segment in a pattern comparable to that described for a midbody segment.

In the anal region ganglion XIX gives rise to two pairs of lateral nerves and as such does not differ anatomically, except in size, from a midbody ganglion. Ganglion XX is the last and also the smallest in the ventral chain. Emerging from the posterior third of this ganglion is a single pair of lateral nerves which is directed caudad. From their position and structural composition they should be considered as homologous to the posterior (second) pair of lateral nerves of a typical ganglion. The branches of this nerve extend into somite XXVI.

The anal ganglia, consisting of eight neuromeres, give rise to eight pairs of lateral nerves. The first pair innervates somite XXVII, the remaining seven pairs innervate the sucker. The last three pairs emerge from the posterior

boundary of the anal ganglia and proceed posteriorly as a group. (See Plate No. II, Figs. 2 and 3).

THE SUBEPIDERMAL NERVOUS SYSTEM

The presence in annelids of a nerve net outside the central nervous system has been previously reported by a number of investigators. Among them, Bristol (1899) refers to the "intermuscular nerve ring," Langdon (1905) and Ogawa (1934–39) include in this system the nervous components of the baseepiment. Hess (1925) in referring to the subepidermal nerve plexus, includes the segmental nerve rings and their peripheral components. In Hess's paper (1925) on *Lumbricus* there is presented the functional as well as anatomical relations of this system to the central nervous system. A preliminary description of the subepidermal system of *Haemopis* is given in a previous paper by the author. Miller (1933). Improvements in invertebrate neurological technique, Miller (1944) have made possible the following supplementary statements relative to this phase of the nervous system in *H. marmoratis*.

The intermuscular nerve rings are segmentally located in the second and fifth annulus of each complete somite. Each nerve ring has, as previously reported, a dorso-lateral and ventro-lateral concentration of nerve cell bodies. Lesser aggregations of cell bodies are found more medially than reported earlier. All of the observed cell bodies of this ring are located within the median longitudinal muscle later. The processes of these cells, combine with the processes of the more peripherally located nerve cells on the baseepiment forming a network beneath the circular muscle layer. This network is connected with the central nervous system through peripheral fibers of the paired lateral nerves.

The segmentally arranged intermuscular nerve rings are joined with each other by anterior-posterior connectives. These connectives have been described as single neurons more or less evenly spaced over the dorsal, lateral and ventral surfaces. Further that the cell bodies of these connectives are located some distance from the nerve rings. (Bristol (1899) for *Nephelis lateralis*). Experimental as well as anatomical evidence verifies the existence of such connectives in *Haemopis marmoratis*. In preparations available to me only dorso-lateral and ventro-lateral connectives are resolvable, and they appear to consist of numerous fibers. The location of the cell bodies of these connectives has not as yet been determined. These connective fibers join with those of the nerve ring in the regions of concentration of cell bodies.

The function of the peripheral system is of utmost importance in understanding certain phases of annelid behavior. I have discussed this aspect at some length in previous papers (Miller 1933–36). An accurate knowledge and an adequate understanding of the nervous system is a necessary prerequisite to the analysis of the behavior of any animal.

THE VISCERAL NERVOUS SYSTEM

This division of the nervous system is referred to by most investigators as the sympathetic system. As far as I am able to determine this portion of the nervous system is associated entirely with the visceral organs, and is not metamerically arranged. The only demonstrable connection with the central system is in the region of the esophageal connectives.

The ganglionic masses of the visceral system are confined to annulus eight. They lie medially in relation to the esophageal connectives and are partly obscured by them. They consist of ganglia located on the dorso-lateral surface of the pharynx and joined by a wide circumesophageal band of connectives. Numerous bundles of nerve fibers extend from this visceral nerve center.

From the ganglionic masses there extends anteriorly numerous bundles of fibers into the buccal cavity. Posteriorly similar bundles extend laterally over the surface of the alimentary tract. In addition to the lateral bundles just mentioned there is a dorsal and a ventral bundle extending from the connectives, the fibers of which form a plexus with the branches of the lateral bundles. The muscular surface of the alimentary tract is in this manner covered with an intricate network of fibers. These fibers are however, of two types regarding their origin. Many of them are continuations from the dorsal, ventral and lateral bundles. Others are the extensions of multipolar nerve cells, the cell bodies of which lie within the muscular wall itself. This system is continous over the entire viscera. (Plate No. II, Fig. 1).

The histology of the entire nervous system of *Haemopis marmoratis* will be presented by the author in the very near future.

Summary

1. This study is concerned with a single species of leech, *Haemopis marmoratis*, (Say).
2. Silver nitrate and gold chloride impregnations provide the basis for nerve differentiation.
3. The illustrations are tracings of projected sections or reconstructions from serial sections.
4. The gross nervous system is composed of four divisions; namely, (1) central, (2) peripheral, (3) subepidermal, and (4) visceral.
5. The leech is divided into three regions; namely, (1) head, (2) body and (3) anal.
6. The central nervous system consists of an anterior cephalization contained in the head region, a series of segmentally arranged ganglia connected by the ventral nerve cord in the body region, and a caudalization found in the anal region.
7. The anterior cephalization represents six neuromeres, and is contained within somites V and VI.
8. Excluding the subesophageal and anal ganglia there are twenty ganglia in the ventral chain. The first eighteen of which are within the limits established for the body region, the remaining two ganglia are in the anal region.
9. The anal or caudal ganglia is composed of eight neuromeres, and is contained within somites XXV and XXVI.
10. The adult body of *H. marmoratis* is divisible into thirty-four segments. Thirty-four ganglia are resolvable and distributed as just indicated.
11. The ganglia of the ventral chain are joined by a pair of nerve trunks extending from the subesophageal ganglia to the anal ganglia. This portion of the central system is contained within the ventral blood sinus.
12. In each ganglion the nerve cell bodies are contained, for the most part, within six primary capsules, two are median ventral, four are dorso-lateral.
13. Extra-capsular nerve cell bodies are found in the central nervous system, within the ventral nerve cord, within the plexiform substance of the ganglia, and within the ganglia but outside the capsule.
14. Six pairs of lateral nerves arise from the anterior cephalization consisting of the brain, circumesophageal connectives and subesophageal ganglia.
15. Extending laterally and in a horizontal plane are two pairs of lateral nerves emanating from each body region ganglion.
16. The anterior pair of nerves is formed by the union of a dorsal and ventral root.
17. From the posterior third of ganglion XX there emerges a single pair of lateral nerves.
18. Emanating from the anal ganglia are eight pairs of lateral nerves. The three posterior pairs emerge as a group and are directed caudad.

19. Intermuscular nerve rings are segmentally located in the second and fifth annulus of each complete somite. With each nerve ring is associated a dorso-lateral and ventro-lateral aggregation of nerve cell bodies.
20. The peripheral nerve net is situated beneath the circular muscle layer, and is connected with the central system through peripheral fibers of the paired lateral nerves.
21. The visceral (sympathetic) division of the nervous system is associated entirely with the visceral organs. There is no indication of segmentation and its only connection with the central system is in the esophageal region.
22. The ganglionic masses of the visceral system are confined to annulus eight. Bundles of fibers emanating from the visceral ganglia extend anteriorly into the buccal cavity and posteriorly over the surface of the viscera.
23. Posterior visceral fibers, supplemented by processes from nerve cells in the viscera form a plexus over the surface of the organs.

LITERATURE CITED

Bourne, A. G. 1884. Contributions to the Anatomy of the Hirudinea. Q. J. Micro Sci. XXIV, pp. 419–508, and XXV, pp. 350–357.
Bristol, Chas. L. 1899. The Metamerism of Nephelis. Ginn & Co., Boston.
Hess, W. N. 1925. The Nervous System of the Earthworm, *Lumbricus terrestris*. Jour. Morph. and Phys., Vol. 40, No. 2.
1925. The Nerve Plexus of the Earthworm, *Lumbricus terrestris*. Anat. Rec., Vol. 31, pp. 335–336.
Krawany, J. 1905. Unterschung uber das Zentral Nervensystem der Regenwurmer. Arb. Zool. Inst. Wien. Bd. 15, s-281–316.
Miller, John A. 1933. Studies in the Biology of the Leech. I. The Subepidermal Nerve Plexus of the Leech. O. Jour. of Sci., Vol. XXXIII, No. 6, pp. 460–463.
1944. Studies in the Biology of the Leech, VIII. Modifications in Neurological Microtechnique. O. Jour. of Sci., Vol. XLIV, No. 4, pp. 177–187.
Ogawa, Fumiyo. 1939. The Nervous System of Earthworm (*Pleretima communissima*) in Different Ages. Sci. Reports of the Tohoku Imperial University, Vol. XIII, 4th series Biol. Sendai, Japan.
Retzius, G. 1892. Das Nervensystem der Lumbricien. Biol. Unters N. F. Bd. 3, pp. 1–16.
Sanchez, D. 1909. Trabajos Del Laboratorio De Investigaciones Biology. S. Raymon Cajol. Tomo VII. Del la Univ. de Madrid.
1912. Tomo X.
Smallwood, W. M. 1923. The Nerve Net in the Earthworm. Proc. Nat. Acad. of Sci., Vol. 9, pp. 95–100.
1930. The Nervous Structure of the Annelid Ganglion. Jour. Comp. Neur., Vol. 51, No. 2, pp. 377–393.
Whitman, C. O. 1888. Some New Facts about Hirudinea. Jour. Morph. No. 2, pp. 586–599.
1891. Description of *Clepsine plana*. Jour. Morph., Vol. XI.
1895. The Metamerism of Clepsine. Festschrift Fur Leuckart.

New Crops for the New World

This book is of paramount importance to those who are interested in tropical agriculture and particularly in the new phases that have developed in Latin America. Dr. Wilson Popenoe has contributed two of the chapters, "The undeveloped field of tropical fruits" and "Cinchona, the fever tree." From his years of experience as plant explorer, research director for the United Fruit Company and now Director de la Escuela Agricola Panamericana, Popenhoe is known far beyond his special field of interest. Edgar Anderson, Albert O. Rhoad, Miriam L. Bomhard, Walter N. Bangham, E. C. Higbee, C. P. Clausen, Arthur Bevan, George E. Adames, Atherton Lee, A. T. Erwin, B. Y. Morrison, P. Honig, and V. C. Dunlap complete the list of contributors to make a book that contains too much carefully worked over data entertainingly presented to fit into a brief review. At no time before have so many biologically trained persons all directed their effort toward the development of western hemisphere crops. The reader can gain an understanding of what the world may expect from teamed effort.—*A. E. Waller.*

New Crops for the New World. Edited by Charles Morrow Wilson. The Macmillan Company, New York, 1945, pp. 295. $3.50.

ON THE STRUCTURE AND MECHANICS OF THE PROTOZOAN FLAGELLUM[1]

HARLEY P. BROWN

Department of Zoology and Entomology, The Ohio State University[2]

The flagellum is an organelle of exceedingly wide distribution among both animals and plants. Among the Protozoa, it occurs not only in all of the Mastigophora, but also in many Sarcodina and Sporozoa during part of their life cycle. The swarm spores and gametes of certain algae and fungi possess flagella, as do the sperm cells of bryophytes, pteridophytes, and cycads. The tail of the typical animal spermatozoon is probably nothing more than a flagellum. The cilia of the ciliate Protozoa and of metazoan ciliated epithelium in all probability represent modified flagella. There is reason to believe that the bacterial flagellum is perhaps the simplest and most primitive manifestation of this organelle. In spite of the apparent diversity of form and function represented in these numerous examples, it seems highly probable that the organization and mode of functioning are fundamentally similar in all.

During the past century numerous investigators have studied the structure and mode of action of flagella, and their conclusions have varied widely. Certain facts have been established, but many points have remained open to question. It has been evident for some time that new techniques would be necessary for the settlement of such questions. Today the electron microscope, with resolution and accompanying magnification far beyond the limits of the ordinary compound microscope, provides such a new technique for the study of minute structures.

Having access to an electron microscope and to pure clone cultures of several flagellate Protozoa, I undertook an intensive study of flagellar structure, the major results of which are embodied in this paper. Flagellar action and flagellate locomotion I investigated by direct observation with the aid of a special technique, by the construction of working models, and by actual underwater swimming experiments.

The work was done at the suggestion and under the supervision and encouragement of Professor W. J. Kostir, to whom I am especially indebted. The electron microscope employed was one constructed by Professor A. F. Prebus, of The Ohio State University, and operated by him and his assistant, Mr. John Dankworth, to both of whom I here express my appreciation for invaluable assistance.

Review of Previous Work on the Subject

STRUCTURE OF THE FLAGELLUM

Until the 1880's or later, the flagellum was generally considered simply an elongate, homogeneous fiber, bearing no appendages, and characterized by weak staining (Bütschli, 1883–7; Klebs, 1892; Dangeard, 1901 b). The flagella were usually depicted as tapering to a point, but Bütschli considered them commonly of equal diameter throughout their entire length, or tapering only slightly at the end.

In opposition to such views appeared the papers of Kunstler (1882, 1889), Loeffler (1889, 1890), and Fischer (1894, 1895). The former described (Kunstler, 1889, p. 408, translation) " . . . a dark axial line resembling a slit or canal, partic-

[1]From a dissertation submitted to the Graduate School of The Ohio State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2]The present address of the author is: Department of Zoology, University of Idaho, Moscow, Idaho.

ularly when the filaments observed are well extended horizontally; in these cases one may often distinguish delicate transverse septa, dividing the axial cleft into rather short sections." (Fig. 1 *a*). The dark sections he considered to represent vacuoles which are separated from one another by septa of the transparent ground mass which surrounds them. He also described a cortical layer or sheath (Fig. 1 *b*, *c*) which was often separated from the ground substance and its enclosed slit.

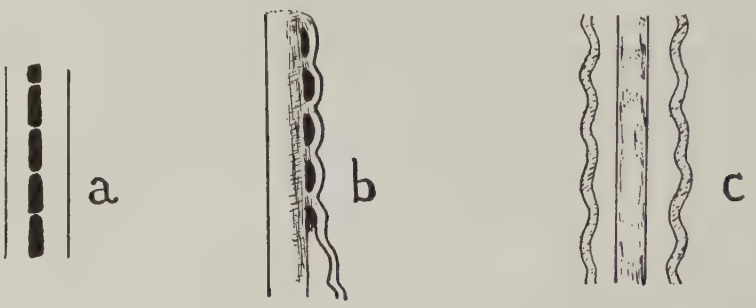


Fig. 1. a. "*Oxyrrhis marina.* Portion of flagellum showing the central fissure sufficiently enlarged to be quite clearly visible, and appearing like a slit or axial groove divided into a series of hollows by delicate transverse septa." b. *Oxyrrhis marina.* "The same flagellum (as *c*) seen at a place where the outer layer is detached only on one side, and only incompletely, in such a way as to form a rather regular sort of corrugation. The cleft which separates it from the internal axis becomes increasingly narrower, and, farther along, even disappears." c. "*Oxyrrhis marina.* Portion of flagellum greatly enlarged and treated with chromic hematoxylin. The undulated outer coat is completely detached from the axial substance." (Redrawn from Kunstler, 1889, Plate XIX.)

To the presence of this enveloping membrane he attributed the weak stainability of the flagellum. The axial substance he considered the more contractile portion. Kunstler also first described an interesting external structure in the flagella of *Oxyrrhis* and *Cryptomonas:* " . . . the attenuated extremity often seems prolonged in a pale filament, thinner than the flagellum and of a different appearance (Fig. 2). The limits of the flagellum proper are distinguished clearly; suddenly, at its tip,

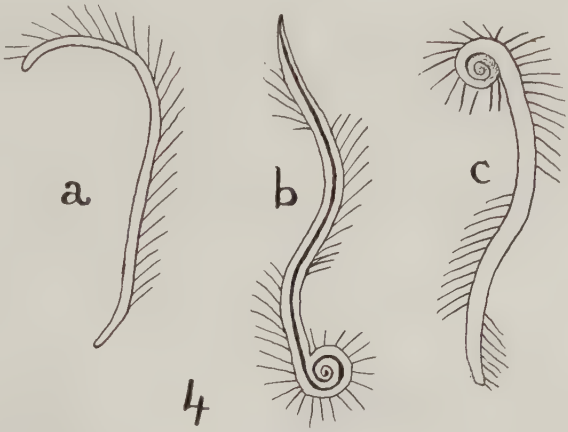
Fig. 2. "*Oxyrrhis marina.* Theoretical figure representing certain flagella. One sees at the end of the true flagellum the pale filament which may be observed in many cases. The same may be seen in *Cryptomonas ovata.*" (From Kunstler, 1889, Plate XX.)

the diameter and appearance change; there begins a transparent filament, hardly visible, very slender, sometimes ending in a slight swelling."

Loeffler (1889) independently described this last-mentioned structure, employing a new technique he was using for bacterial flagella. He suggested that the thick basal part might be a sheath, from which extends the delicate protoplasmic fiber in the manner of a pseudopod, slightly swollen terminally. The apical swelling, he postulated, might function as a tactile organ. Loeffler also discovered on the

flagellum of a monoflagellate (perhaps *Oikomonas*) two dense rows of extremely delicate hairs, projecting almost at right angles to the axis of the flagellum, one along each side. (See Fig. 3). He did not investigate the possibility that in life the hairs actually might cover the filament instead of being arranged in two rows, and in drying be flattened down laterally, producing the impression of two rows of hairs. At any rate, he had described and figured photomicrographs of two previously unknown appendages, and outlined his method in detail.

3



4

FIG. 3. *Monas* sp. ? The first published figure of a flimmer-flagellum, from a mordant-stain preparation designed for bacterial flagella. (Redrawn from Loeffler, 1889, Plate II.)

FIG. 4. *Euglena viridis*. a. "Cast-off flagellum . . . still completely stretched out; untwisted, with the hairs on one side . . ." b. "A cast-off flimmer-flagellum rolling up, partially swollen and therefore with an apparent axial fiber." c. "A cast-off, rolling-up flagellum which had previously become uniformly swollen and consequently shows no such apparent structure" (as Fig. 4 *b*). From preparations of undiluted culture dried upon cover glasses in about one-half hour, and treated with a modification of Loeffler's mordant-stain technique. (Redrawn from Fischer, 1894, Plate XI.)

Fischer (1894), using a modification of Loeffler's technique, made a careful study of the flagella of *Euglena viridis*, *Monas guttula*, and species of *Bodo*, *Chlorogonium*, and *Polytoma*. He confirmed Loeffler's findings and discovered that in *Euglena* the flagellum bears but a single row of hairs (Fig. 4), while that of *Monas* has two opposite rows. He termed these flagella Flimmergeisseln, or ciliated flagella. Since "flimmer" appears to be a convenient word for the structures, and does not carry any obvious implications in the English language, I shall hereafter employ the term "flimmer-flagellum" in reference to any flagellum bearing hair-like projections along one, two, or all sides.

Whip-flagella, or Peitschengeisseln, Fischer found in *Bodo*, *Chlorogonium*, and *Polytoma*, and described as follows (p. 230, translation): "The whip-flagellum consists of a thick homogeneous stalk, previously thought to be the entire flagellum (the only part visible in unstained specimens) and, arising from its tip, a very delicate whip-thread 2–3 times as long as the stalk. This thread is slung about like a coach-whip by the strokes of the basal stalk."

Fischer observed in *Euglena* what he concluded to be an artifact arising from incomplete swelling: " . . . the central, not yet swollen part of the flagellum appears as a denser, more strongly stained axial fiber, while the outer, swollen part, seems a less dense, more weakly stained ground substance." (See Fig. 4 *b*). Further, "In *Polytoma* and *Bodo*, a relatively commonly seen granular structure of the basal part of the whip is likewise only a result of the technique. This flagellar structure,

which agrees with that described by Kunstler, is to be regarded in this light" (i. e., as an artifact). (For further discussion of the granular appearance, see Gelei, 1926.)

Fischer studied in detail the processes of flagellar disintegration, whereby he made his observations on the artificial nature of the granular or alveolar appearance of certain flagella. He figured in detail many examples of all the above-mentioned types, and discussed each matter thoroughly. In spite of the fact that he could demonstrate no natural inner structure in either whip- or flimmer-flagellum, he stated (p. 204): " . . . yet there is no doubt that the apparently homogeneous fiber must possess a very fine structure made up of definitely arranged particles (micelles), toward which point the twisting of the flagellum, its rolling up, and the arrangement of the flimmer-hairs."

Regarding the arrangement of the flimmer, Fischer assumed that they must beat in unison, since, on any given flagellum, all the flimmer project in the same direction.

For years flimmer-flagella were generally considered artifacts, perhaps the result of a final wriggle and smear, or of chemical action (Plenge, 1898; Dangeard, 1901b; Bütschli, 1902; Schuberg, 1905; Korschikov, 1923; Günther, 1928). On

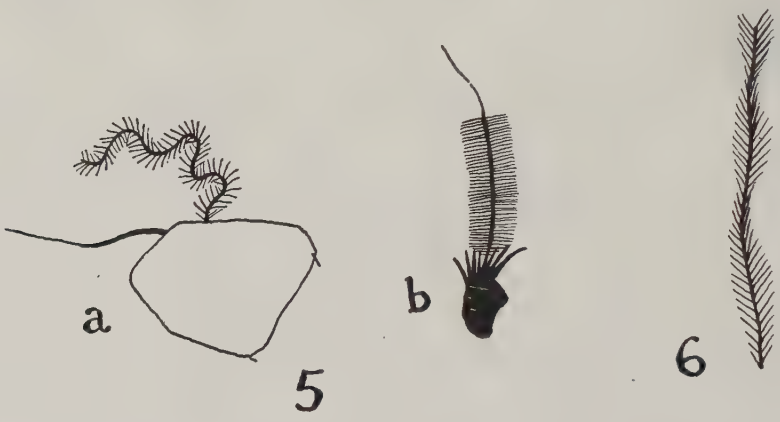


FIG. 5. a. "*Synura uvella* Ehrenb. A cell from the colony with flimmer- and whip-flagella." b. "*Salpingoeca* sp." (Redrawn from Petersen, 1929.)

FIG. 6. Schematic flimmer-flagellum showing appearance which would be expected to occur in a twisted flagellum if the flimmer existed in two lateral rows, rather than all over the surface of the flagellum. This sort of appearance has not been observed in specimens, although many of the observed flagella have probably been twisted. (From discussion and diagram of Vlk, 1938.)

the other hand, most of these workers confirmed the existence of whip-flagella, and in many cases observed such structures in living specimens. During the latter part of the period covered by the above references, several investigators were, however, confirming the reports of Fischer and Loeffler. Petersen (1918) demonstrated the two flagella of *Synura uvella* to be of different types (Fig. 5*a*): " . . . the one of whiplike form, the other a pinnate flagellum showing two opposite rows of little secondary filaments, and resembling a feather; it is to be presumed, however, that these [filaments] extend from all sides of the primary filament." (Translation from summary in French.) Petersen also found flimmer-flagella in *Uroglena* and *Dinobryon*. In a later paper (1929) he expanded his observations to a large number of forms, some possessing only whip-flagella, some only flimmer-flagella, and some having one of each. He also described a combination whip-flimmer-flagellum in Craspedomonads. (Fig. 5 *b*). The distribution among the Protozoa of consistent flagellar types fits well into the accepted scheme of classification.

Mainx (1928), though failing to demonstrate flimmer-flagella in several species reported to possess them, confirmed their presence in *Euglena viridis* and figured excellent photomicrographs of the flimmer-flagellum of *Phacus pleuronectes*. His most exacting attempts to observe flimmer on the living flagellum failed. In consideration of their extreme regularity along one side of the flagellum, their presence with or without fixation, etc., he concluded that they could hardly be artifacts. He considered them, however, not motile, as Fischer had assumed, but rather simply as devices serving to increase the flagellar surface.

Petrova (1931) employed flagellar types in the solution of a taxonomic problem.

Deflandre (1934), using a very different method of preparation, confirmed the presence of whip- and flimmer-flagella in many organisms, and considerably expanded the list of species studied.

Vlk (1931, 1938) has perhaps treated the problem most thoroughly, expanding and organizing the described types, and tabulating his results. For a more thorough treatment of this phase of flagellar structure, the reader is referred particularly to Vlk, 1938, or to Deflandre, 1934. Vlk even succeeded in observing flimmer (in two distinct rows) on the flagellum of active, living specimens of

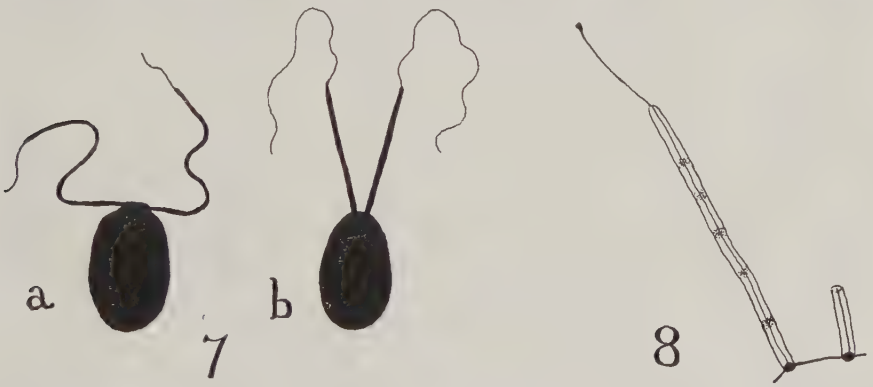


FIG. 7. a. Whip-flagella of *Chlamydomonas moewusii*. b. Whip-flagella of *Chlamydomonas sestinensis*. (Redrawn from Gerloff, 1940, to show variations in length of whip.)

FIG. 8. *Cyclidium glaucoma*. Silver preparation. Greatly enlarged cilium showing the axial fiber extending from the basal granule through and beyond the "contractile" sheath, and ending in an enlargement. The dark areas in the sheath are irregularly spaced and even lacking in some of the cilia nearby. A silver line connects adjacent basal granules. (Redrawn from part of Text Figure 34, Klein, 1929.)

Mallomonas acaroides, a species having an unusually large flagellum. These flimmer are described as having a length about 6 times the diameter of the flagellum proper. He was able to demonstrate these living flimmer-flagella to members of the institute staff, including Professor A. Pascher.

Unfortunately, Kudo (1939), in his brief treatment of the flimmer-flagellum, gave all credit to Vlk but misrepresented Vlk's diagram of a two-row flimmer-flagellum (Fig. 6). The error is certainly unintentional, but misleading. This diagram, which Kudo used to illustrate a two-rowed flimmer-flagellum, was originally intended by Vlk (1938, p. 486) to represent a situation which *should* occur in twisting if the flagellum actually bears two distinct flimmer rows. But such a situation had never been observed. Instead, the flimmer of these forms usually appear somewhat as in Fig. 3 or Fig. 5 *a*. In other words, Vlk used it as a probable indication that the flimmer, in most organisms at least, are distributed on the surface of the flagellum like the hairs on a dog's tail, and not just in two lateral rows.

Recognition of the whip-flagella has been rather general, and they seem almost universally accepted today. Gerloff (1940) figures the whip-flagella in many species of *Chlamydomonas*. A considerable variation in relative length of lash and stalk may be noted (Fig. 7). Many authors (e. g. Awerinzew, 1907; Minchin, 1922) consider the lash or end-piece as a portion of an axial fiber extending beyond the enveloping sheath of protoplasm and membrane. Cilia are now commonly considered to have a similar structure (Klein, 1929). Klein seems to agree with Loeffler's hypothesis that the terminal swelling of the lash (Fig. 8) may have a sensory function.

Figure 8 (or 10 *a*) will also serve for a discussion of the generally accepted concept of the internal structure of a flagellum. Most textbooks and articles on the subject agree to the presence of an axial fiber surrounded by a protoplasmic

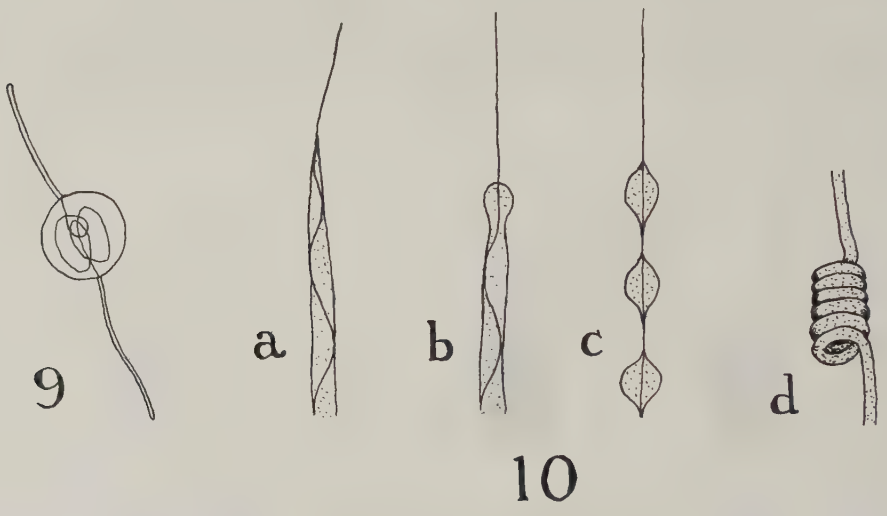


FIG. 9. Flagellum of *Peranema* in which the axial fiber and outer membrane are intact, although part of the intermediate substance has swollen and formed a knot or coil of the axial fiber. (Redrawn digrammatically from Korschikov, 1923, Fig. 1.)

FIG. 10. a-c. Flagellum of *Chilomonas paramecium* undergoing disintegration (half-schematic). The protoplasmic portion flows together into droplets, exposing the axial fiber and allowing it to straighten. d. *Ceratium tripos*. Part of contracted longitudinal flagellum. (Redrawn from Awerinzew, 1907.)

sheath. Beyond that there is much less agreement. A few consider the axial core as the major contractile or vibratile portion, the sheath serving as a supporting structure (Rosskin, 1922; Calkins, 1933). The vast majority think differently. (Bütschli, 1902; Schuberg, 1905; Awerinzew, 1907; Goldschmidt, 1907; Williams, 1907; Erhard, 1910; Hamburger, 1911; Minchin, 1922; Korschikov, 1923; Doflein, 1929; Klein, 1929; Kudo, 1939; Hyman, 1940).

The flagellum (or its axoneme) usually arises from a basal granule (blepharoplast), apparently essential to the continued function of the organelle. In certain forms the flagellum has two roots and basal granules (e. g. *Euglena;* Wager, 1899; Hall and Jahn, 1929). For information on other modes of attachment, see Prowazek, 1903; Goldschmidt, 1907; Schouteden, 1907.

Regarding the interrelationships among axoneme, surrounding plasm, and outer membrane, there are interesting reports, based upon studies of disintegrating flagella, which throw light upon the subject. Fischer (1894) made extensive

observations on the coiling or rolling-up of flagella, noting that the process might begin at either end of the flagellum or at any point in between. Korschikov (1923) confirms Fischer's description of flagellar "aggregation" (Figs. 4 *b, c;* Fig. 9), summarizing his observations as follows (translation from German summary): "The axial fiber and the outer membrane are the most resistant structural components of the flagellum. In the 'aggregation' of the flagellum, the intermediate substance is first destroyed. In the corresponding region the membrane becomes swollen. In the course of this process, the axial fiber is drawn into the swelling and forms there a ball or coil (Fig. 9). Afterward, however, when this blister of the flagellar membrane is destroyed, the axial fiber is freed and stretches again in length."

Awerinzew (1907) and Hamburger (1911), describing flagella undergoing destruction, state that the "protoplasmic" portion of the flagellum rounds into globules which proceed to flow or withdraw toward the base of the flagellum, either leaving the axoneme exposed and straightened out (Awerinzew) or telescoping it (Hamburger). (See Fig. 10.) All of these facts tend to indicate an elastic, upporting axoneme.

Úlehla figures, but barely mentions, an interesting flagellar structure (Fig. 15), reminiscent of Kunstler's diagrams, but seen in living flagella.

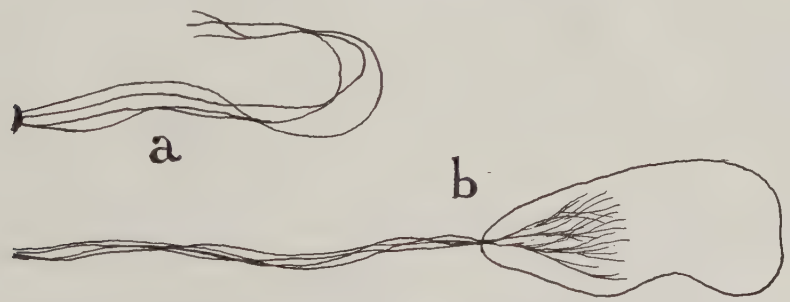


FIG. 11. a. "The uncoiled fibrils of the flagellum of Euglena." b. "Euglena with the fibrils of the flagellum branching out into a system of rootlets in the protoplasm of the body." "This structure is demonstrated with ease by subjecting a flagellum to slight pressure." (Drawn from Dellinger, Figs. 1, 3.)

Roskin (1922, 1923) postulates and Korschikov (1923) asserts that the axial fiber consists of a great number of thin fibrils. Koltzoff (1903) stated that in certain pteropods the cilium "consists of several fibers which are encased in a common fluid plasma layer . . . the inner fibers running to the basal body . . . " Schmitt, Hall, and Jakus (1943) figure cilia (*Frontonia*) and flagella (*Trichonympha*) frayed into 9–11 fibrils.

Dellinger (1909), who figures fibrils in both cilia and flagella (Fig. 11), states: " . . . the flagella of Euglena, Chilomonas and Spirillum are composed of four spiral filaments." He also depicts the axial filament in the pseudopod (axopod) of *Actinosphaerium* (Heliozoa) as fibrillar. A similar structure is described in the heliozoan axopod by Rosskin (1925). For discussions of the possible homology between such pseudopods and flagella see Minchin (1922), Goldschmidt (1907).

The flagella of many bacteria entwine in a tuft and function as a unit, in some cases even becoming encased with a glutinous mass which keeps them bound together (Reichert, 1909; Úlehla, 1911; Metzner, 1920; Pijper, 1941; Hutchinson and McCracken, 1943). This situation is perhaps analogous to that occurring in cirri or in the "cilia" studied by Gray (1922) and Carter (1924), in which a number of units capable of beating individually are united into a single organelle.

Sperm tails display a fibrillar axis and an enclosing sheath, and have been more exhaustively studied than flagella. Furthermore, they are probably homologous to the protozoan flagellum, although the details of the homology are not yet settled (Dangeard, 1901a; Alexeieff, 1924; Grassé, 1926; Duboscq and Grassé, 1933). Subsequent to the work of several investigators, Ballowitz stated in 1890 that many types of sperm tails frayed into 2–4 subfibrils. In his paper of 1908, he figures tails fraying into as many as 18 subfibrils. Koltzoff (1909) produces interesting figures of sperm tails, including such as Fig. 12, showing a sheath of helically wound gel fibrils. Recently the electron microscope has made possible more minute studies. Several articles (Baylor, Nalbandov, and Clark, 1943; Harvey and Anderson, 1943; Schmitt, Hall, and Jakus, 1943; Schmitt, 1944) figure micrographs of sperm tails showing, in frayed parts, 9–12 fibrils (Fig. 13). According to

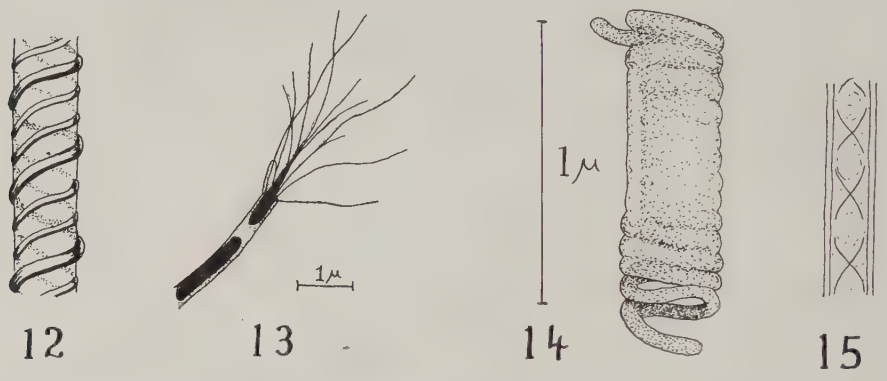


Fig. 12. Flagellum (tail) of *Planorbis* sperm, showing outer helix of gel fibers. (Redrawn from Koltzoff, 1909, Fig. 13.)

Fig. 13. "Frayed tail of bull sperm showing subfibrils."

Fig. 14. "Portion of sheath from human sperm tail fragmented ultrasonically, showing helical structure." (Redrawn from electron micrographs; Schmitt, 1944, Figs. 1, 2.)

Fig. 15. "*Gonium pectorale*. A portion of a momentarily resting flagellum, showing the structure of the flagellum. The helical lines proceed . . . uninterruptedly to the tip. (Schematic)." (Redrawn from Úlehla, 1911, Fig. 55.)

Schmitt, these fibrils are smooth, parallel, 300–500 A wide, and run the full length of the tail. Occasionally even finer subfibrils are seen. "In mammalian sperm tails the bundle of fibrils is surrounded by a sheath, the major component of which is a closely wrapped helical fibril, having a thickness also in the range of 300–500 A (Fig. 14). When the tails are fragmented by ultrasonic radiation, portions of this helix can be seen, appearing like miniature solenoids." (Schmitt, 1944, p. 36.)

MECHANICS OF THE FLAGELLUM

In the past, little actual correlation has been made between structure and function in flagella. Many authors have recorded types of flagellar movement and general movement, but relatively few have attempted to analyze the forces or mechanisms involved. First, let us enumerate the types of movements described. Úlehla (1911, pp. 727–728) classifies them as follows:

(1) Monad type. Flagellum long, cylindrical, flexible; beats forward in loops (spirals) grading into flattened waves (i. e., undulations; a given point on the flagellum follows an elliptical path).

(2) Chrysomonad type. Similar to above, but with shorter and stiffer flagellum.

(3) *Euglena* type. Long, twisted, ribbon-like flagellum; beats laterally in loop-shaped waves.

(4) *Bodo* type. (Trailing) flagellum with long end-piece, rather stiff; beats in flattened waves.

(5) *Clostridium* (a bacterium) type. Flagellum long, spiral, rather stiff; slow waves from base to tip.

(6) Chlorophycean type.

a. Swarmer type. Flagellum short, cylindrical, rather strong, with the basal part more flexible; functions as an oar (in sculling?), swinging around as if contracting as a unit.

b. *Pandorina* type. Flagellum longer, more flexible, often somewhat flattened; beats with a spiral lateral stroke.

Ulehla hardly considered the paddle-stroke as such, though this type of movement is described by many, being reported in flagella by Plehn (1904), Rosenbusch (1908), Doflein (1916), Kofoid and Swezy (1920, 1923), Krijgsman (1925), Nigrelli (1929), Kirby (1943); in cilia by Gray (1922) and Carter (1924), among others. Such a lash-movement is mentioned also by Pütter (1903), Goldschmidt (1907), Schindera (1922), and Grassé (1926). In some such cases, it evidently occurs in flagella which ordinarily undulate. In all of these cases, the flagella concerned were located anteriorly.

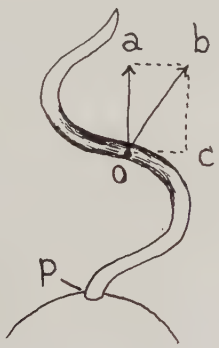


FIG. 16. See text for explanation.

The second major type of movement executed consists of undulations, usually occurring in two planes (i. e., a spiral). Reports of this type may be found in Bütschli (1887), Kunstler (1889), Goldschmidt (1907), Laveran and Mesnil (1907), Friedrich (1909), Reichert (1909), Bancroft (1913), Kofoid and Swezy (1919), Schindera (1922), Grassé (1926), Entz (1928), Petersen (1929), Lowndes (1936, 1941, 1943, 1944, 1945).

The third type, not listed above, is simple conical gyration, reported by Keysselitz (1906), Metzner (1920), and Entz (1928), and by McDonald (1922), in cilia.

Primarily because of Krijgsman's detailed paper, which was based on studies of *Monas* sp. under darkfield, most textbooks today emphasize the paddle-stroke. It presents a clear-cut, diagrammatic, relatively simple-looking movement, familiar in our own swimming stroke and similar to that commonly depicted for cilia. The filament is held rigid during the effective stroke, but is flexible during the recovery stroke.

The concept of progression by a spiral turning of the flagellum has led a painful existence because of Bütschli's analysis of the situation as he saw it (Bütschli, 1887, p. 857 et seq.). Delage and Hérouard (1896, pp. 306–307) treat his explanation thus

(translation): "Suppose that the flagellum assumes the form of an elongate helix (as indicated in Fig. 16), and that this helix begins to turn clockwise (as seen from the base). At any point such as *o* the pressure on the water would create a force *ob* perpendicular to the flagellum, which could be resolved into two others, the vertical one *oa* determining its rotation about its axis. It is to be noted that this rotation is in the opposite direction to that of the flagellum. If the helix turns in the reverse direction, the animal will go backwards. In the case of a helix wound to the left, there would be likewise progression forward for a certain direction of rotation and backward for the opposite direction. . . . All that is very well, but Bütschli does not note that the movement he describes supposes an agency met in our mechanical instruments, but never among living beings. In order that the body and the flagellum might turn indefinitely in opposite directions about the point *p*, it would be necessary that the mode of union between them be that of a pin stuck through a card, capable of turning freely in its hole, its union with the card assured by the head which could not go through the hole." But this leaves Delage and Hérouard with the problem of explaining matters. They do so by going into great mathematical detail concerning the possibility of a forward component produced by conical gyration, concluding this to be impossible by direct action. However, by its indirect action, i. e., by the rotation produced in the body, they think to have found the answer; namely, that since the flagellum is firmly attached to the body it must rotate with the body, and being held in a corkscrew shape, will thus screw the body and flagellum through the water.

Reichert (1909) noted the forced aspect of this scheme and pointed out that, in his experience, the facts argued against such a mechanism. Further, he states (translation): "The necessity for their relatively cumbersome explanation of flagellar movement appears to have been a result of a misconception of Bütschli's idea. For Bütschli postulates that an opposing rotation of body and flagellum is not feasible in his view that the lines of contraction move about the flagellum. If the lines of contraction on the flagellar surface circle to the right, the body will turn to the left, and so will the flagellum, about its axis. . . . It is only necessary that the lines of contraction move correspondingly faster about the flagellum, so that its turning with the body is equalized or offset. Then the same effect would be achieved as if this turning of the flagellum did not occur at all."

Delage and Hérouard are not the only ones to misinterpret Bütschli's idea.

Reichert explains it, and the physics involved, quite clearly. Then, in his summary he states: "Reversal of movement is accomplished quickly by polar-flagellated bacteria, for they simply reverse the flagellar rotation (as Reichert and Bütschli postulate that they should), or by bipolar-flagellated bacteria, whose flagella take turns (those at the other end begin beating). In peritrichous bacteria, on the other hand, reversal occurs slowly. Movement must first cease for a moment, and the flagella assume an opposite orientation to the body." But he is inconsistent, for he also states: "*a*. The flagella (in bacteria) are always wound in a right spiral (clockwise) and rotate always to the right (as seen from the rear), i. e., the screw-like lines of contraction wind around the flagella to the right. *b*. The body always rotates to the left. *c*. The flagella are usually directed backward during locomotion. In the spirilla this occurs too, whether the flagellum arises at the anterior or posterior pole." If they "*always*" rotate in one direction, how can they "*simply reverse*" this rotation? Further, if the flagellum is directed backward during locomotion, whether the flagellum be at either end of the body, does this not indicate that the flagellum is assuming a different orientation, rather than that it is merely rotating to the right or to the left? For, if it simply reversed its direction of rotation in order to back up, it would then pull the body instead of pushing it, and would not be directed backward. The error doubtless lies in the assumption that the organism moves backward by simply reversing the flagellar rotation. Indeed, the theory itself seems disputed by these facts.

Gray (1928, p. 35) gives a fine explanation of the mechanics involved in undulating flagella, the gist of it being that there is a series of waves passing along the flagellum in the same direction, requiring no recovery stroke. The propulsive power " . . . is equivalent to that which would be produced by projecting along the length of the flagellum a series of 'humps' of the same form as the waves, the velocity of the humps being made equal to the velocity of movement of each wave. If the waves pass from the base of the flagellum to its tip, the organism is driven forward in front of the flagellum; if the waves pass from the tip to the base the organism is drawn forward with the flagellum in front. If the waves pass along the flagellum in one plane there will be no force tending to rotate the animal on its axis: if, however, the waves pass round the flagellum as well as along it the organism will rotate." This is well and good, but it fails to fit a couple of facts; namely, that most flagellates move flagellum-foremost, and that the waves of transmission along the flagellum have only been demonstrated to progress from the base toward the tip (Lowndes, 1936, 1941 a and b, 1943, 1944 a and b, 1945 a and b).

To date, Lowndes has made the most complete and accurate analysis of the situation in a number of flagellates. His conclusions are based upon observed facts and specific data, backed up by high-speed cinema photomicrography (for techniques, see Lowndes, 1935, 1944 a). Too much of previous explanations was based upon pure speculation and rationalization. Lowndes (1941 b) has demonstrated that the flagellum ordinarily *pushes* the organism, even though situated at the anterior end, by being directed backward. In this connection it is interesting to consult Úlehla's figures, many of which indicate the same. This appears true even for *Peranema*, the organism classically cited as having a "tractellum," or flagellum which pulls the organism forward, presumably by a wave starting at the tip. Lowndes shows that, in all cases studied, the wave invariably progresses from base to tip. This fact is an upsetting one to most of the previous theories. Among other workers reporting this type of action are Kunstler (1889), Friedrich (1909), Úlehla (1911), Bancroft (1913), and Schindera (1922). Of these, Úlehla and Bancroft give accounts almost precisely like that of Lowndes concerning the locomotion of *Euglena viridis*.

In his later papers, Lowndes elaborates a new concept, previously mentioned by him, but misunderstood by such workers as Barker (1943). In the summary of one of these papers (Lowndes, 1944 a), he states it as follows:

"1. The primary function of the flagellum in a monoflagellate organism is to produce both rotation and gyration of the organism about a certain axis which constitutes the main direction in which the organism is swimming.

"2. The mechanical principle by which the organism is propelled is simply that of the inclined plane which is caused to rotate. In other words it is that of the screw or propeller.

"3. Since the disturbances or waves pass down the flagellum in the form of a spiral they produce two distinct components. It is the resultant of these two components which causes the tip of the organism both to rotate and gyrate.

"4. So long as this rotation and gyration is maintained it will supply the necessary force for the propulsion of the organism.

"5. The flagellum itself may or may not produce a forward component. If it is more or less swung out at right angles, as in *Menoidium*, it will produce no forward component but if it is swung back, as in *Euglena*, it will do so."

Hence, it is the *rotation and gyration of the body* of the organism which is considered to produce the major component of force. Others have observed, naturally, that in organisms bearing obvious helical external structures, rotation of the body would produce a component of force. For instance, Günther (1928) correlated the speed of several species of *Euglena* with spiral ridges on the periplast and flagellar length. Thus, of two species having flagella of approximately the same length, one having strong spiral ridges progresses at about three times the relative

speed of another having very insignificant ridges. But no one previous to Lowndes, so far as I know, had suggested the possibility that the *gyration of the body itself* might produce locomotion.

Propulsion by simple conical gyration is what Delage and Hérouard thought they had proved, by mathematics, to be impossible. Metzner (1920) showed this to be erroneous, both in theory and in practice. By means of experiments on wires rotating in water, he demonstrated that a simple conical gyration produces a pulling force which reaches maximum at an angle of 20–23 degrees from the axis of rotation. He even went so far as to state (translation): "Among flagellates, the simple conical gyration predominates; the organism 'sucks' itself along through the water by means of the flagellum." It should be noted that he considered only gyration of the flagellum, not gyration of the body. Such conical gyration of the flagellum is reported by Keysselitz (1906) and Petersen (1929). It is also described by Lowndes (1944 b), who performed experiments similar to those of Metzner. However, Lowndes contends that this mode of locomotion is incompatible with high speed, as the flagellum could not be maintained in such a forwardly-directed position. In rapidly moving organisms, the flagella must beat laterally to the rear.

In this same paper, and in his most recent one (1945 b), Lowndes renders untenable a number of misconceptions which arose long ago through misinterpretation of the original data, and have grown or continued from textbook to textbook. One such item regards diagrams of *Monas* swimming, taken from Krijgsman (1925). I shall not discuss these errors here. As Lowndes points out, Krijgsman's observations were excellent records of the situation he was studying, *but* that situation was not representative of the free swimming of the organism. When the animal is freely swimming, the mechanism is quite different from the paddle-stroke observed by Krijgsman, being similar to that described by Lowndes for *Euglena*, and the rate of progression is about ten times as rapid as that given by Krijgsman. Locomotion is primarily brought about by the rotation and gyration of the body of the organism. (See also Lowndes, 1945 a.)

As for the question of the precise nature of what goes on within the flagellum—it remains a question. For years, writers were occupied with a controversy as to whether the flagellum was an active unit or simply an appendage like a whip, manipulated from the base or cell body. The latter concept was eventually discarded as such, but lingers in part in the question whether or not the flagellum can execute any movement after losing connection with the cell. Various workers (e. g. Klebs, 1892; Entz, 1926) have reported the phenomenon; others have flatly asserted such to be impossible (Verworn, 1890; Korschikov, 1923). However, the work of many investigators has indicated that the flagellum is an active unit. Gray (1928) enumerates good reasons for assuming such, and Lowndes (1936, 1941 b, 1945 b) proves it with photographic, quantitative evidence. Granting, then, that the flagellum does possess the ability to move, by what mechanism does it effect this motion?

From the versatility of movements and also " . . . because its movements at times cannot be explained by simple mechanical laws," Krijgsman (1925) concluded that the flagellum " . . . must be of complicated construction." Engelmann (1881) and Ballowitz (1890) assert that movement is associated with fibrillar structure, which, as we have seen, is evidently present. Erhard (1910) and Lowndes (1941 b) regard the transmission of the stimulus as a surface phenomenon, not connected with the axoneme. Such statements are generalities, to be sure, but, if accepted, eliminate such theories as that of Gurwitsch (1904), which postulates a rapid streaming of protoplasm into and out of the flagellum, acting in antagonism to the elasticity of the axoneme. Heidenhain (1911) proposed a "theory of smallest waves" to explain movements: these waves may travel along one side of the filament, they may vary in length and effect, they may vary in extent, they

may vary in path, frequency, and rhythm. They are evidently quite versatile waves. So far as I know, they have been forgotten. Pütter (1903) discussed Engelmann's theory and decided that it required too many spiral fibrils of different pitch and direction. Verworn (1915) stated what he considered the basic principle involved (translation): " . . . a contractile side contracts from the cell body outwards and thereby stretches the opposite side, which latter in the expansion phase, by its elasticity, returns the filament to its rest position. According to the opposing relationship of the contractile to the passively stretched substance, there results a beat in one plane or in a complicated form." This does epitomize the principle employed by most of the theories. For a discussion of the theories, see Gray (1928).

Viewing the matter from a more generalized standpoint, but with fundamentals in mind, Gray (1928) and Schmitt (1944) liken the process involved to that of muscle, and consider the basic phenomenon to result from a change of distribution of water molecules "between polar groups in fibrous proteins and ionogenic groups in the environment" (Schmitt). In other words, if the proteins in the fibers along one side of the flagellum suddenly take on great numbers of water molecules at the expense of the opposite side of the filament, the filament (flagellum) will tend to bend toward the side losing the water. This sudden affinity for water by the protein might be due to a change in the degree of ionization of the protein molecule, caused by a local production of acid. Should the acid then be neutralized, the water would again be liberated (Gray). Or it might be that the protein fiber consists of folded molecular chains which fold up further upon the addition of water, thus contracting the fiber. At any rate, we get glimpses of the possibilities.

Original Investigations

Structure of the Flagellum

Materials and Methods

It was considered desirable to experiment briefly with the more productive methods described by other workers in the study of flagellar structure. Such techniques as those of Gicklhorn (1921), Kater (1929), Allen (1936), and Smyth (1944) are useful procedures for general study or mere observation of flagella, but contribute relatively little to a detailed investigation of the type here undertaken. On the other hand, the papers of Loeffler (1890), Fischer (1895), and Deflandre (1923) describe techniques of primary importance in the development of our knowledge of whip- and flimmer-flagella. McClung (1937, pp. 141–145) presents Loeffler's method and may be more readily available to persons attempting to duplicate some of the work done on flimmer- and whip-flagella. Vlk (1938) describes his modifications of the Loeffler technique. Petersen (1929) points out the possibilities for failure and sources of error, emphasizing the extreme delicacy required in preparation. Several authors (e. g. Bütschli, 1902; Korschikov, 1923) have failed to achieve success with the mordant technique.

Such contributions as this paper may offer to the knowledge of flagellar structure are made possible through the use of the electron microscope. Further probings with the light microscope promise but little, as the possibilities have been rather carefully covered. For previously employed methods of preparation of specimens for electron microscope examination, see Marton (1941, 1943), Morton and Anderson (1942), Mudd and Anderson (1942), Richards and Anderson (1942), Prebus (1944), Williams and Wyckoff (1944, 1945 a, b), Claude and Fullam (1945), and Porter, Claude, and Fullam (1945). Some of the techniques described are adapted to specialized situations, but they bring out various points of importance in the preparation of biological material for electron microscope study. Among the most important of these, perhaps, are the removal of salts and other matter which might crystallize upon or otherwise obscure or mar the specimens, and the thinness

of film plus specimen, which should total less than 50 mμ in thickness. A good discussion of the limitations of the electron microscope for biological work in general is given by Richards and Anderson (1942). For basic principles and general limitations of the instrument, see Hillier and Vance (1941), or such popularized books as Burton and Kohl (1942) or Hawley (1945).

Dellinger (1909) and Porter, Claude, and Fullam (1945), among others, consider osmic acid the best killing and fixing reagent for retaining original appearances. I have found it extremely useful in this work. Over a dozen different procedures have been employed, the most successful of which have all involved the use of osmic acid. Formalin as a fixing reagent has apparently resulted in excessive clumping, but this has not been thoroughly investigated. Other major difficulties in the preparations were due to too many or too few organisms on the film, crystallization, loss of flagella in centrifuging, and, most of all, disruption of membranes or films under electron bombardment.

The principal organisms studied were *Astasia klebsii* Lemmermann, *Euglena gracilis* Klebs, *Ochromonas variabilis* Meyer, and *Chilomonas paramecium* Ehrenberg which were obtained from pure cultures maintained in the Protozoology Laboratory at The Ohio State University.

The film and screen upon which specimens were to be mounted was prepared as follows (slight modification of method employed by Prebus):

(1) 300 mesh/inch bronze screen is cleaned by immersing in dilute HCl and washing a number of times in distilled water.

(2) Rectangular pieces about 6 x 18 mm. are cut from the screen and kept dust-free (e. g., in a Petri dish).

(3) A vessel (e. g., a Pyrex pie plate) of about 10-inch diameter with a level rim is filled to the brim with distilled water.

(4) The water is saturated with amyl acetate.

(5) A drop or two of 2% collodion (cellulose nitrate) in amyl acetate is allowed to fall upon and spread over the surface of the water.

(6) After a few seconds a film is left, subsequent to the evaporation of the solvent. This film is then swept off by means of a glass rod, in order to clean the surface of the water. This may be repeated if it seems advisable.

(7) A drop of the collodion solution is allowed to fall an inch or less onto the surface of the water.

(8) A rectangular piece of screen, held in an artery clamp, is immersed in the water near the rim of the vessel, moved over beneath the film, and raised directly up. If parts of the film do not tear neatly around the edge of the screen, they may be severed with a needle tip by running it along the edge of the screen.

(9) Excess water is removed from the screen and film by touching the droplets with a folded corner of filter paper.

The film thus mounted on the rectangle of screen may be used immediately or allowed to dry before having specimens placed upon it. Immediate use seems preferable.

Of the techniques employed in the preparation of specimens, the following were most successful:

1. (a) Place 4.5 ml. of a pure culture of the desired organisms in a centrifuge tube.

(b) Add and mix 0.5 ml. of 2% osmic acid or 2% osmic acid in 1% chromic acid.

(c) Add 5 ml. of distilled water and centrifuge 30 seconds.

(d) Decant, then refill to 10 ml. mark with distilled water.

(e) Re-suspend organisms by rolling tube between palms of hands.

(f) Centrifuge 30 seconds.

Repeat (d) to (f) inclusive once more.

(g) Decant, then add distilled water to raise meniscus to the 0.5 or 1.0 ml. mark. Again suspend organisms by rolling the tube vigorously between the palms.

(h) Place a drop of the liquid containing the concentrated, washed organisms upon film-screen and allow the organisms to settle for 30–60 seconds.

(i) Carefully remove some of the excess liquid from the top of the drop by touching to it the folded corner of a piece of filter paper.

(j) Allow to dry, then examine under compound microscope for promising specimens. An average of about 1 organism per "pane" or mesh of the film is the optimum concentration of specimens. If much more numerous, the film does not support them when under electron bombardment. If much less numerous, the specimens are too difficult to find in the electron microscope.

(k) Place screen, specimen side down, upon a rectangular piece of heavy onion-skin paper which is conveniently larger than the piece of screen. With a screen rectangle of 6 x 18 mm., a paper rectangle of about 12 x 30 mm. is of convenient dimensions. (See Fig. 17.)

(l) Flatten the screen against the paper and secure it there by means of small pieces of Scotch tape (thin, transparent, cellulose adhesive tape).

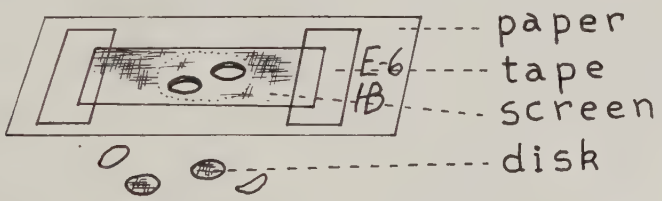


FIG. 17. See text for explanation.

(m) Label one end of the paper for identification and reference to procedure, as in Fig. 17: "E-6, HB."

(n) Place in a Petri dish or between concavity slides to keep perfectly clean, and remove to the electron microscope laboratory.

(o) Using a die constructed for the purpose, punch out circular disks of screen about 2 mm. in diameter.

(p) Separate disks of screen from those of paper and examine under compound microscope (on a clean slide).

(q) Select promising disks for electron microscope examination.

2. The following method, since it does not involve centrifugation, subjects the flagella to less drastic treatment, but requires a greater initial concentration of organisms in the culture. Various modifications of this method were employed, some eliminating step (b).

(a) Fill depression of a concavity slide with an especially rich pure culture.

(b) Add several drops of Gicklhorn's stain (made by adding about 5 drops of concentrated NH_4OH to 50 ml. of 0.05% methylene blue solution).

(c) Let stand about 1 minute and add 1 drop of 2% osmic acid in 1% chromic acid solution.

(d) Allow organisms to settle for 30–60 seconds, then touch the surface of the liquid with a folded corner of filter paper to remove excess liquid.

(e) After removing a large part of the liquid as above, carefully replace this liquid with distilled water, by means of a pipette.

Repeat (d) and (e) at least 5 times, to wash the specimens of acid and of salts, extraneous material, etc.

(f) Observe under a compound microscope (16 mm. objective) while drawing organisms into a micro-pipette. They will be concentrated in certain areas at the bottom of the drop.

(g) Deposit by means of this pipette as many of the organisms as possible in a medium-sized drop upon a film-screen mount.

(h) Allow organisms to settle, then remove excess liquid as in (d).

Continue as in (j) to (q) inclusive, technique No. 1.

Both of the above techniques were essentially developed by the author in the course of this work, as were various other less successful ones.

Results

Upon the development of satisfactory techniques, it was possible to demonstrate a number of details relative to the structure of the flagellum. Some of the more satisfactory and instructive of the electron micrographs obtained are shown in the appended plates. In viewing these photomicrographs, the reader should bear in mind that the objects and details perceptible are not rendered so by staining, as in preparations for the light microscope, but are visible because they have diffracted or absorbed varying proportions of the electrons passing through them. Thus a body appears dark because it is denser or thicker, or both, than the surrounding substance, and not because it has been differentially stained. From a careful examination of these plates, the following points may be observed:

(1) Each flagellum is of approximately uniform diameter throughout its entire length (Plates 4; 6; 9; 10; 11; 12).

(2) Each flagellum consists of a denser axial core (axoneme) and a less dense sheath surrounding the core (Plates 2 to 8 inclusive).

(3) In the flagella of *Euglena* and *Astasia*, the axial core appears to consist of two closely approximated fibers of equal size (Plates 1 *B*; 2 *A*; 4; 5; 6; 9).

(4) The sheath appears to contain or to consist of a coiled fiber which encircles the axial core in the form of a helix (Plates 1 B; 3 *A*; 12).

(5) The flagella of *Euglena* and *Astasia* bear, along one side, what appears to be a single row of delicate filaments extending from the sheath. The length of the filaments is about 5 or 6 times the diamater of the flagellum, or 1.5 to 2.0 μ. (Plates 1 *B*; 4; 9).

(6) The long flagellum of *Ochromonas* bears similar filaments along both (all ?) sides (Plates 11; 12).

(7) The flagella of *Chilomonas* bear no such lateral filaments (Plate 10).

It is possible that the ground substance or intermediate substance which might be expected to occur between axial fiber and sheath, perhaps comprising a large part of the bulk of the living flagellum, has escaped in most of the specimens shown. Consideration of Plate 7 might lead us to this conclusion, assuming the darker upper portion of the flagellum to represent a region which, somehow, had not yet lost the inner plasm. Perhaps the swollen appearance in Plate 8 is due to an accumulation or exudation of such matter during the drying of the specimen. From Plate 7 we also get an indication that the lateral filaments might possibly be due to the escape (and subsequent coagulation) of plasm from a lateral series of minute pores. In view of the work of Vlk, who demonstrated flimmer upon a living flagellum, this explanation might be held to apply to the possible origin of flimmer in the living organism. In such a case, complete coagulation might not occur until death.

A second possible explanation for the appearance shown in Plate 7 is that a portion of the flagellum might have contracted, bringing the coils of the helix into closer approximation and producing the denser and thicker appearance seen in the upper portion of the flagellum shown. It is difficult to determine precisely what actually happened to produce this effect, and the interpretation of such an appearance must remain uncertain for the present.

Upon inspection of the plates, it will be noted that, as stated in (1) above, the flagella depicted are of approximately uniform diameter throughout their entire length. Little, if any, tapering occurs at either extremity. Emmel, Jakob, and Gölz (1942) report the same condition in electron micrographs of *Leishmania donovani*.

Of the other points listed above, (2) simply substantiates the generally-accepted notion of flagellar structure. (4) serves to emphasize the similarity between the protozoan flagellum and the mammalian sperm tail, as described by Schmitt and others. (5), (6), and (7) corroborate, by an entirely new technique, the findings of a number of workers, extending somewhat the knowledge of the details.

Unfortunately, many of the best "shots" were lost, due to the rupture or the supporting film or the sudden curling up of flagella under the impact of the electron bombardment, before photographs could be taken. However, the fact that a flagellum, even though flattened out against the film, in a high vacuum (0.00001 to 0.0001 mm. Hg.), and dead for many hours, can retain within itself the potential ability to tear itself loose and curl up like a watchspring, may be significant. Similar coiling during the disintegration of flagella has been described by several investigators (e. g., Fischer, 1894), but never with violence like this.

The photographs included in this paper were, of course, selected to bring out various points. Plate 6 shows an entire flagellum, about 13 μ in length, extending from the body of the euglena. The flagellum is seen to extend, not from the anterior tip of the organism, but from a point slightly lateral and posterior to the tip, where it emerges from the mouth of the gullet. Plate 6 also shows the naked axoneme, where the supporting film has torn, snapping the flagellum off near its base. Plate 2 *A* probably represents a flagellum lying adjacent to the body, and extending away from it near the posterior tip of the body. Plate 2 *B* gives an indication of the relative strength or durability of the axoneme and the sheath, the axoneme being apparently stronger and more elastic.

Plates 4 and 9 display especially well the single flimmer-row on the flagellum of *Euglena*, and represent flagella prepared by different techniques. Plates 11 and 12 show flimmer along both sides of the long flagellum of *Ochromonas*, which might, in life, occur either in two opposite rows or all over the surface of the flagellum like the hairs on a dog's tail. It will be noted that the flimmer do not occur in the neatly regular rows depicted by previous workers. This may be accounted for by the fact that these specimens have been centrifuged several times in preparation, a drastic measure not employed by previous workers. Such a disarrangement should be expected.

Plate 9, in portions depicting twisting, shows clearly the two major fibers of the axoneme in the flagellum of *Euglena*.

Plate 3 *A* indicates the helical structure in the sheath rather well. Such plates as 2, 5 and 7 also suggest this helical structure. Other plates, in which the sheath does not stand out perceptibly from the axoneme, may be of interest in that there occur at more or less regular intervals along the sides of the flagellum dark spots which may represent the helix closely appressed to the axial core. Plates 1 *B*, 9, 11, and 12 show such indications. If this is the correct interpretation of such appearances, the pitch of the helix on the flagellum of *Ochromonas* is considerably greater than that of *Euglena* (i. e., the coil is less tightly wrapped, forming longer spirals). Further, if this be the correct interpretation, the spots shown in Plate 9 will be of especial interest. Many of them are lighter in the center, indicating that the coiled fibril may be hollow or tubular.

Plate 1 *A*, which is an electron micrograph of a diatom, is included to show our method of computing sizes. Large numbers of this type of diatom, a species of *Gomphonema*, were collected several years ago, cleaned, and calibrated. Lateral transverse rows of pores may be seen extending from a median longitudinal solid

area like the barbs in the vane of a feather. The pinnate rows along one side of the median line are usually more nearly perpendicular to the axis of the median line than are the rows along the other side of the line. The average distance between consecutive rows is about one-third of a micron. We obtain measurements by averaging the distances between every fourth row. This average is about 1 μ. After taking a series of micrographs at a given magnification, a diatom is photographed at the same magnification, in order to provide a scale for measurement. This method and the initial calibration of the diatoms were worked out by Dr. Prebus.

MECHANICS OF THE FLAGELLUM

Observations on Living Organisms

Flagellar action is, in most cases, very difficult to observe in normally-moving or freely-swimming creatures. The flagellum is hard enough to see when still, and when in active motion is beyond the capabilities of the human eye. For this reason, most studies on living flagella have been made on organisms under abnormal conditions. They have been chilled, anesthetized, compressed, placed in viscous media, or simply observed in the latter stages of approaching death, when the water beneath the cover glass was drying up. Realizing that normal activity is hardly to be expected under such circumstances, yet assuming that certain basic phenomena should remain constant, I have made a few observations under some of the above-mentioned conditions.

The most convenient method I have found for rendering flagellar motion visible involves the use of methyl cellulose (Methocel, Dow Chemical Co.). A drop of 10% solution of this substance is mixed on a slide with a drop of culture, then a cover glass added (Marsland, 1943). The resulting mixture, of rather high viscosity, slows down the strokes of flagella or cilia, and also is of a very different refractive index from water, such organelles becoming much more easily visible than in water.

Among the structures observed by this method were the flagella of *Peranema trichophorum*, *Euglena gracilis*, and *Trichonympha* sp., the undulating membrane of a trichomonad from the gut of *Reticulitermes flavipes*, and the cilia of *Paramecium* sp. The optical system employed included a Spencer 4 mm. objective (N.A.—0.85) and a 20 $\times$ Planoscopic ocular, with a resulting magnification of about 880 $\times$. In every case, the wave impulse traveled from the base toward the tip, in a spiral course, producing rotation of the tip. All of these observations directly confirmed certain conclusions of Lowndes (see historical review). In *Euglena* the flagellum was usually directed back more or less along the body. I was somewhat surprised to find this sort of movement in cilia, as I expected to see the paddle-stroke described by Gray and others. However, the cilia were observed to alter the direction of their strokes quite readily, beating forward, directly outward, or backward (and toward or away from the observer). The spiral, flagellum-like stroke or undulation was most conveniently observed when the cilia were beating directly outward, or away from the body surface. The cilia beating thus created a current away from the body. I have wondered whether this spiral undulatory stroke in cilia might be due to the greater density or viscosity of the medium employed in these experiments. Cinematic photography of such cilia under more nearly normal conditions should aid in clearing up the matter. Alverdes (1922) made an extensive study of ciliary movement in several species of *Paramecium*, *Stentor*, etc. He ascribed to the cilia considerable versatility of movement. He also described interesting experiments on the shedding and regeneration of cilia by *Paramecium*. He kept the organisms in a 0.1% solution of chloral hydrate for about 48 hours, then transferred them to fresh water, and, after 3 to 9 hours, observed the regeneration of the cilia. The cilia began beating when only stubs.

This technique, combined with good cinematic photomicrography, might produce very interesting results.

I have observed, without altering the medium in any way, flagella in colonies of *Volvox* which seemed capable of performing almost any movement possible for a filament attached at one end. Since the colony was probably suffering under adverse conditions, the movements probably were not normal, but they certainly served to emphasize the versatility of movement possessed by the flagellum. To quote again from Krijgsman's summary (translation): " . . . its movements at times can not be explained according to simple mechanical principles." It is too easy to agree with him.

Experiments on Locomotor Mechanisms.

"ARTIFICIAL FLAGELLATES." In order to test the forces produced by rotating and gyrating objects, a device was worked out as shown in Fig. 18, whereby structures comparable both to bodies and to flagella of flagellate organisms could be studied in this connection. Originally it was devised for comparison with a flagellum, but when the significance of Lowndes's hypothesis became apparent to me (upon the receipt of his later papers), it was extended to a study of body gyration.

This hypothesis, it will be recalled (see historical review), suggests that *the major component of force producing the forward locomotion of a monoflagellate results from the rotation and gyration of the body of the organism*, and not directly from the action of the flagellum. Consequently, a model was constructed in the shape of a sample protozoan (e. g., see Fig. 22) in order to test the locomotor effect produced by the rotation and gyration of such a body. As figured in the accompanying diagram, *B* represents this body, with *a* representing its axis. The arrows encircling the axis of forward progression *A* serve to indicate the path of gyration of the body axis *a*. All of the rest of the diagram below the body *B* is simply included to show how the rotational and gyrational force is applied to *B*.

Thus in Fig. 18, *A* represents the axis of gyration and progression about which the cork body *B*, with axis *a*, is caused to rotate and gyrate. In the diagram, the body is gyrating clockwise as seen from the base or rear. (Throughout

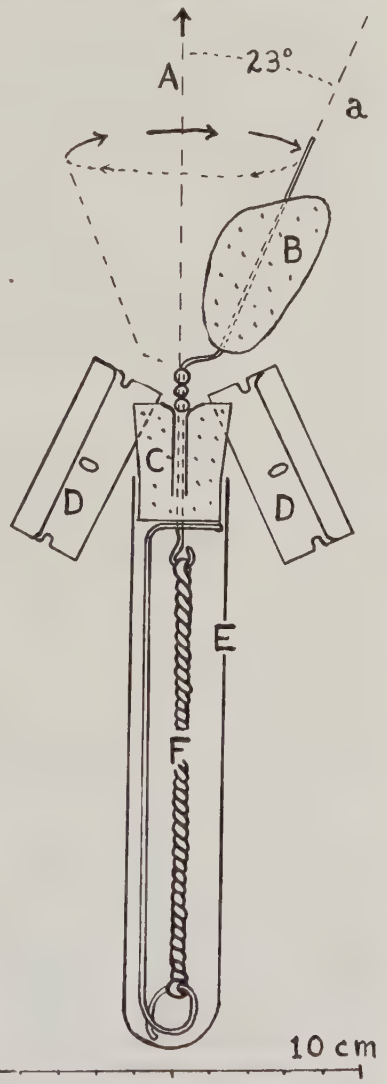


FIG. 18. Diagram of the "artificial flagellate." *B* represents a model of a monad body, which rotates and gyrates about its axis of progression, *A*. *a* represents the body axis. The lower part of the diagram merely indicates the apparatus employed to impart to the body *B* its rotational and gyrational force. See text for explanation and discussion.

this paper, when the terms clockwise or counter-clockwise are employed, reference is made to the rotational path of the distal gyrating extremity as viewed from the apex of the gyrational cone.) A heavy rubber band, *F*, twisted in the desired direction, produces the rotation which, in turn, causes the part of the wire bent out of line to gyrate. It was often found desirable to use two rubber bands, in order to obtain greater speed and force. Beads below the bend in the wire served as bearings, and turned in the funnel-like flared end of a metal tube which was inserted in the cork stopper *C*. Since the rotation and gyration of *B* caused the base (*C*, *D*, *E*, etc.) to rotate and gyrate in the opposite direction, it was found necessary to reduce such rotation considerably; otherwise, the rubber band rapidly became untwisted. Two razor blades, *D*, inserted in the cork parallel to axis *A*, served as fins or keels in reducing rotation of this portion of the system. In order to balance the system to a specific gravity slightly above 1.0, water was added to test tube *E* in the necessary amounts. Paraffin was found to be less satisfactory in achieving this balance. The angle (β) between *A* and *a* was altered simply by bending the wire. The body, *B*, may be replaced by other objects of diverse shapes and sizes.

1. Currents produced in fluids by gyrating structures. Experiments were performed with the "artificial flagellate" using smoke, in air, and minute suspended particles, in water, to observe the currents produced by the gyration of *B*.

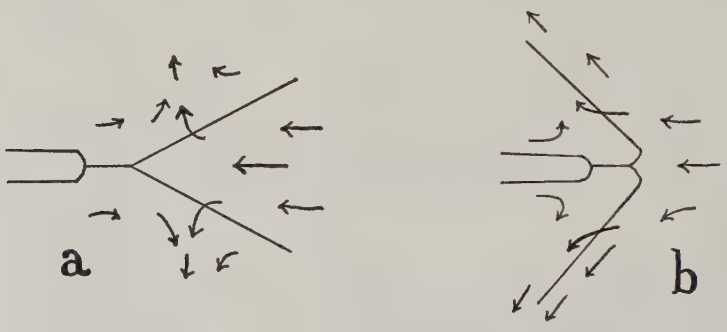


Fig. 19. Currents produced by gyrating wires in water. (Redrawn from Metzner, 1920, Figs. 3, 4.)

The results of these experiments, which were not extensive, were in accord with those of Metzner (1920). In brief, if the path of gyration forms a simple cone, fluid is drawn into the cone primarily from its base, departing near the apex (Fig. 19). Thus a major current is produced toward the base of the cone in the surrounding medium. If the body were free to move, it would progress toward the base of the cone. Lowndes (1944 b) has also demonstrated rather fully the action of similar objects. For theoretical considerations as to the mode of function, see Metzner, (1920, pp. 53–58).

2. Locomotion of gyrating systems in water. A number of experiments were performed to determine the velocity achieved by the "artificial flagellate" in water, relative to the shape and position of the body *B*. In each case, both clockwise and counter-clockwise gyrations were tested, in order to eliminate such factors as the possibility that the razor blades (*D*) might be acting as screws or propellers. These experiments were carried out in a greenhouse tank (200 x 80 x 60 cm.).

It was found that when structures much larger than *B* were placed on axis *a* to gyrate, the rear end of the test tube *E* was caused to gyrate considerably. Since this would exert a force in the opposite direction, it was objectionable. By placing

around the test tube a fairly close-fitting, rigid jacket, this gyration may be minimized. A coarse screen or hardware cloth is perhaps best, although a test tube slightly larger than *E* was first used for the purpose. In the latter case, there is too much difficulty involved in the movement of water to fill the space vacated by the progressing system. In the set-up as shown in the diagram, the gyration of the test tube was sufficiently unimportant to be neglected in the gross observations being made.

Velocity was measured horizontally and vertically. The latter measurements represent much freer motion on the part of the "flagellate," but are more difficult to obtain under the conditions encountered. The "organism" is balanced so that it sinks rather slowly. Then, wound up, it is held down, allowed to gyrate a few times, and timed on its way up (its stable position is in the vertical axis). For the most part, it was timed through a distance of 20 cm. A stop watch was used in all cases. Horizontal runs were made by placing the test tube in some such jacket as mentioned in the preceding paragraph and holding the jacket steady in the horizontal plane. By the nature of this set-up, such runs were confined to a distance of 5 or 6 cm. Since, in every case, *B* was buoyant, the gyration was uneven in this plane. Another factor necessitating brief runs, both horizontally and vertically, is that the force producing the gyration diminishes rapidly as the rubber bands untwist. In consideration of these, and perhaps other conditions, it is obvious that the measurements are necessarily inaccurate. To assume an error of $\pm 10\%$ would be optimistic. However, the figures are at least indicative, and have some value thus.

Relative speed, as here employed, equals distance traversed by the organism in one second divided by the length of the gyrating body. Thus, if the body *B* were 5 cm. long and the system moved at 10 cm./sec., the relative speed would be $10 \div 5$, or 2. Among Protozoa which have been actually timed for rate of swimming, the relative speeds vary from 0.25 (*Euglena terricola*, Günther, 1928) or less, to 40.0 (*Monas stigmatica*, Lowndes, 1944 b, 1945 a) or more.

With a long body (*B*), 14 x 1 x 1 cm., at 1 to 1.1 gyrations/second, traveling horizontally out of a vial, with angle β at 23°, the maximum constant velocity observed was 1 to 1.2 cm./sec., representing a relative speed of about 0.08. Using the same set-up, but with angle β greater than 90° (see Fig. 19 *b*), the maximum velocity was 0.33 cm./sec. This condition hardly corresponds to any natural one.

With a short body (*B*) as shown in the diagram, 4.7 x 3.7 x 2.4 cm., at an estimated 6 gyrations/second, traveling vertically, with angle β at 23°, the maximum constant velocity was 25 cm./sec., representing a relative speed of about 5. With angle β at 15.2°, a maximum velocity of 33 cm./sec. was observed. This was under ideal conditions and was never quite duplicated. It represents a relative speed of 7, the highest obtained in these experiments.

To test the forces produced by a flagellum undulating in a helix or spiral, the body, *B*, was made in such a shape by bending a wire and coating it with 1.5 to 2.0 mm. of paraffin. The form was approximately that shown in Fig. 16, but with a less complete pitch. The complete pitch would be about 20 cm. The flagellum rotated at about 18 turns/sec. With the flagellum rotating counter-clockwise, the system moved forward at 34 to 38 cm./sec. With the flagellum rotating clockwise, the system moved backward at 20 to 22 cm./sec. If a living flagellum beats from the base outward, the latter is the only type of motion compatible with the system. In order for an actual flagellum to execute a movement similar to the former, the wave of contraction would have to begin at the tip of the flagellum. If this occurred, it would constitute a "tractellum." It probably does *not* occur in nature.

From our experiments with the "artificial flagellate," we learn that the mere rotation and gyration of a body in water can provide sufficient force to produce rapid forward locomotion of the body. This greatly strengthens the hypothesis advanced by Lowndes (1944 a).

UNDERWATER SWIMMING. These experiments test, in a fashion, the strength of the "pull" exerted by gyrating structures. In contrast with flagellate bodies, but like the flagella themselves, the gyrating objects do not necessarily rotate. The arms of the swimmer serve as the gyrating structures. The rate of gyration is approximately 1/sec. The gyrating portion is 60 cm. in length. Figures on velocity are computed from the distance traversed in about one-half minute. The body weight of the swimmer is about 130 lbs. or 59 kg. In each case, enough air has been expelled from the lungs to allow the body to sink to the bottom of the pool. All experiments were performed by the author. Timing was done by an observer with a stop watch.

(1) The body is horizontal, with one arm extended horizontally forward and gyrating, e.g. clockwise, in a relatively narrow cone. (Fig. 20 *a*) Result: the body moves horizontally forward, rotating counter-clockwise, in this case. Velocity, 10+ cm./sec. Relative speed, 0.16+. Total distance progressed, 10 ft.

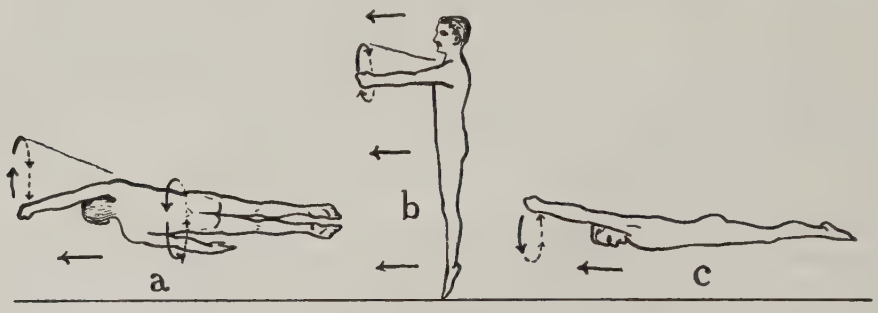


FIG. 20. Underwater swimming experiments. See text for explanation.

(2) The body is vertical, with both arms extended horizontally forward, the right gyrating clockwise, the left counter-clockwise or *vice versa*. (Fig. 20 *b*) Result: the body moves horizontally forward, not rotating. Velocity, 10+ cm./sec. Relative speed, 0.16+. Total distance progressed, 10 ft.

(3) The body is horizontal, with both arms extended horizontally forward, the right gyrating clockwise, the left counter-clockwise. (Fig. 20 *c*) Result: the body moves horizontally forward, not rotating. Velocity, 33 cm./sec. Relative speed, 0.55. Total distance progressed, 32 ft. Reversing the gyrations, the velocity is considerably less, being at most about 23 cm./sec., with a relative speed of about 0.39. Total distance progressed, 22 ft. This may well be due to an unintentionally weaker stroke, as it is more difficult and tiring to the experimenter.

(4) The body is in the same position as (3), but both arms are gyrated in the same direction. Result: the body rotates in the opposite direction at approximately the same rate, little forward movement being accomplished.

(5) The body is in position (3), but the arms are pendulated or swung back and forth in one plane, instead of being gyrated. Result: no detectable forward component.

Minor components are omitted in this consideration, for the sake of simplicity. In many cases they are results of the musculature of the arm.

Other experimental positions were tried, but contribute no additional significant data and are hence omitted.

The results of these experiments provide additional evidence in support of Lowndes's hypothesis. They serve further, however, to show that rotation of the gyrating object is not necessary to the production of a forward component. *Mere gyration of an object* (an arm or a flagellum) *can produce an effective locomotor force.*

Discussion

From the historical review given in this paper, it is apparent that a considerable mass of knowledge has been accumulated on the subject of flagellar structure, but that, in large part, the data have not previously been assembled and organized. Certain phases of the subject, to be sure, have been well summarized. For instance, Vlk (1938) treats whip- and flimmer-flagella as such rather thoroughly, but neglects the internal structure. However, he is not to be criticized for this, as little new knowledge has appeared relative to the matter within several decades. The subject has awaited a new technique which could permit of more minute investigation. The electron microscope provides this new angle of attack through its much greater resolution and magnification. This paper presents the results of the first intensive study of the protozoan flagellum employing the electron microscope.

By means of this instrument, we find that the flagellum, at least in the forms studied, consists of a dense fibrillar axial region and a surrounding sheath of much less density. This much has been previously accepted, though never before so clearly demonstrated. Just how many fibrils there are comprising the axoneme, I cannot tell. The number probably varies to some extent. The sheath seems to contain a fiber which encircles the axoneme in the form of a helix. This sort of composition of axoneme and sheath appears to be in close agreement with the structure of mammalian sperm tails as described by Schmitt and others, but has never before been described in the protozoan flagellum, unless the brief remarks of Úlehla (1911) be considered such. Unlike the sperm tails, certain flagella possess lateral or terminal external extensions of the sheath. These structures have been recorded since 1889, but are not yet universally accepted by protozoologists. Several electron micrographs of the lateral structures in question (the flimmer) are included in this paper (e. g., Plates 4, 9, 11, 12). Of course, such pictures constitute no conclusive proof of the nature, or even natural occurrence, of the flimmer. But they certainly contribute to the evidence in favor of their acceptance as normal structures, rather than as artifacts. If Vlk had had at his disposal the equipment of Lowndes (1935), Harvey and Loomis (1931), or Pijper (1940), perhaps the question might have been settled for the most skeptical by the actual photographing of flimmer on living flagella. However, the facts that they have been demonstrated by a number of basically different techniques, on dried or moist flagella, following various fixatives or none at all, and that they are amazingly constant in appearance, position, etc., place the overwhelming weight of evidence in favor of the view that they represent normally-occurring structures in many types of organisms. The presence and type of flimmer or whip comprise valuable taxonomic characters, and will doubtless be so considered when they are more readily demonstrable.

There are a number of devices as yet untried in the study of these structures, some of which should certainly contribute to our knowledge of their nature. I mention them in the hope that someone, to whom they may be available, may be interested in furthering this study. By means of the shadow technique of Williams and Wyckoff (1944, 1945 a, b), the flimmer should be made obvious. A study of living flagella might be made with the ultra-violet microscope (Lucas, 1930, 1934; Schmitt, 1939; Lavin and Hoagland, 1943), whereby twice the resolution of the ordinary microscope might be obtained. Phase difference microscopy (Richards, 1944) offers interesting possibilities in the study of living flagella, yielding the benefits of stains and fixing reagents, as does the ultraviolet microscope, without requiring the use of such deleterious agents. (That is, it serves to differentiate structures and substances which we commonly bring out only by staining.) Fluorescence microscopy (Ellinger, 1940; Metcalf and Patton, 1944) also gives promise, especially, perhaps, in the study of flagellar motion. If nat-

urally fluorescing flagella occur, these would be ideal for such a study, but a fluorescent vital stain such as Fluorescein might serve well.

It is interesting to view the results of Kunstler and Fischer in the light of details brought out in electron micrographs. If the reader will again refer to Fig. 1, he may notice certain similarities to some of the plates. The interpretation of the appearances, however, is rather different. The axial canal of Kunstler is probably the axoneme. Plate 7 shows, in places, an apparent breaking up or segmentation of the axoneme into pieces resembling the "vacuoles" of Kunstler, as seen in Fig. 1 *a*. Figs. 1 *b* and *c* are reminiscent of the helix of the sheath as seen in the electron micrographs, the corrugations depicted by Kunstler probably representing the surface appearance of the structures. It must be borne in mind that Kunstler had at his disposal nothing like the resolution and magnification of the electron microscope; his observations were remarkably good, in consideration of this fact. Segmentation of the flagellum may somehow be produced by the action of osmic acid, in combination with other factors. Kunstler had used this reagent in his preparations, as have I. Gelei (1926) showed that such appearances resulted from certain reagents, among them osmic acid in combination with other chemicals.

Fischer apparently found a continuous axoneme in many flagella, but took great pains to demonstrate or argue that it was but an artifact. He, too, did a good job with what he had, and presented a rather convincing argument. However, in the light of evidence since brought forth, including much in this paper, it is highly probable that he was mistaken in his interpretation of at least the nature of the axial fiber.

Regarding the structure of the flagellar filament, Plate 7 presents an interesting picture, which, as Dr. Prebus points out, may well represent at the denser end the natural appearance of the flagellum. Here the helical structure in the sheath is evident, but the inner axial fibers are obscured by the density of the surrounding matter within the sheath. In the less dense portions of this filament we see what is perhaps the result of a loss of material from the sheath or intermediate substance. This lighter portion corresponds to the appearance of most of the flagella shown, and brings out the internal structure, but may thus represent a state of disintegration of the flagellum.[3] The ground substance which may have escaped is precisely that which is considered to be the contractile portion of the flagellum by many workers. For my part, I find it easier to attribute the major contractility to the fibrillar structures remaining.

In this connection, I might mention a possible mechanism for the progress of the contractive wave which has not heretofore been suggested, so far as I know. It has no basis in experimental evidence, and is simply proposed for consideration. Since the flagellum appears to have a fibrous core which is encircled by a helical fiber, as represented diagrammatically in Fig. 21, it is possible that the spiral fiber transmits the impulse. If along this fiber passed a wave of chemical change, comparable to that in a firecracker fuse or a nerve fiber, perhaps releasing H ions wherever it passed over the axial fibers, it could stimulate local contraction in such parts of the axial fibers as might be contiguous with it. In conjunction with twisted axial fibers (Dellinger, 1909), this might account for undulatory movements of a spiral nature. In such organisms as *Peranema*, the axial fibrils might be stiffened or less sensitive in the basal portion, thus accounting for the fact that normally only the distal part shows obvious activity, whereas, if stimulated to a

[3] A second possible explanation for the appearance shown in Plate VII is that a portion of the flagellum might have contracted, bringing the coils of the helix into closer approximation and producing the denser and thicker appearance seen in the upper portion of the flagellum shown. It is difficult to determine precisely what actually happened to produce this effect, and the interpretation of such an appearance must remain uncertain for the present.

greater extent, the entire flagellum is thrown into undulations. Also, if the axial fibrils along one side should be less reactive, the resultant stroke might be paddle-like. With a bit of imagination, this hypothetical mode of functioning can be fitted to most observed facts. At least, it fits the facts better than any other system proposed, so far as I have been able to discover. However, it remains purely speculative.

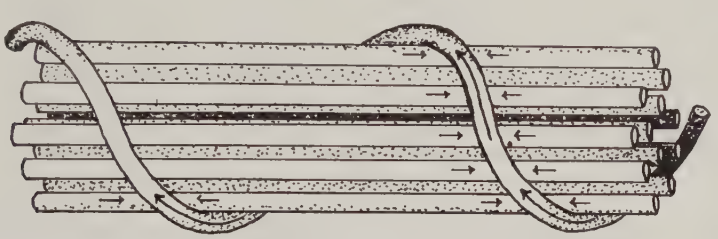


FIG. 21. Diagram illustrating hypothetical path of impulse in flagellum. See text for explanation.

Likewise in the realm of speculation to an extent, but also of interest, in a different way, is the appearance of the axial core of the flagellum of *Euglena* (Plates 2 *A*; 5; 9). There seem to be two equal fibers running side by side, which are displayed especially well where the flagellum (or merely the fibers) is twisted. These fibers are probably composed of yet finer fibrils (see Dellinger, 1909), though such are not evident in the plates. Now it happens that *Euglena* has a bifurcate flagellar root. Two distinct branches, arising from two separate basal granules, unite in the gullet at about the level of the stigma (eyespot) to form the single flagellum (Wager, 1899, et al). Hartmann and Chagas (1910), in consideration of the bipartite root, considered *Euglena* as derived from a biflagellate. It seems probable to me that the two fibers within the flagellum definitely strengthen such a view, and might be regarded as the two ancestral axial fibers or axonemes in close association.

Another interesting source of speculation is the relationship between the bacterial flagellum and that of the protozoon. Electron studies have been made on many bacteria. Among authors reproducing numerous electron micrographs of bacterial flagella are Piekarski and Ruska (1939) and Mudd and Anderson (1944). According to these and other authors, the flagella average from 14 to 50 mμ in diameter, and often occur in clumps or tufts, as they do in the spirochete *Treponema* (Mudd, Polevitsky, and Anderson, 1943). Their precise nature still appears doubtful. The suggestion has been made (e. g., Polevitsky, 1941) that they may be hollow tubes, but Mudd and Anderson (1942, p. 106) find no support for this idea in cases which superficially present such an appearance. Mudd, Polevitsky, Anderson, and Chambers (1941) figure an electron micrograph of *Bacillus subtilis* with the protoplasm shrunken away from the cell wall. On the other hand, Mudd and Anderson (1944) figure *Vibrio cholerae*, showing a "single polar flagellum, which seems to traverse the cell wall to join the bacterial protoplasm." At any rate, bacterial flagella, whatever may be their exact nature, and even though they may not arise in a tuft, often entwine to function as a unit and may even remain adherent, forming a filament composed of many fibrils and perhaps encased in a gelatinous (?) sheath. (See historical review for references.) Now recall the flagellar or sperm tail structure with its 9 to 12 fibrils, each 25 to 50 mμ in diameter, ordinarily encased in a sheath. The possibility certainly is suggested that the ancestral protozoan flagellum may have been derived from such a permanently united clump of fibrils as occurs in some of the bacterial forms. Should this prove

to be the actual phylogenetic origin of the protozoan flagellum, the concept of the fibrils as contractile units, rather than as mere elastic rods, would be definitely strengthened. However, the fibrils might well serve both as contractile and as supportive structures.

With regard to the mode of functioning of the locomotor flagellum, my observations and experiments confirm the conclusions reached by Lowndes. The living flagella observed beat in spiral undulations, the waves of contraction always progressing from base to tip. My experiments with an "artificial flagellate" and with underwater swimming demonstrated that sufficient force is produced by the simple conical gyration of a body to account for rapid locomotion toward the base of the cone. Fig. 22 and its legend describe the propulsive mechanism as conceived by Lowndes. I have never, to my knowledge, watched the swimming of this particular organism, but I have observed other similar ones. From my own experiments and the work of Mr. Lowndes, I have no doubt that the gyration of the organism *could* account for a relative speed of 40 (that given by Lowndes for *Monas stigmatica*), if the body gyrated with sufficient rapidity. But in the forms I have observed, it is difficult to imagine how the body gyration alone could produce such

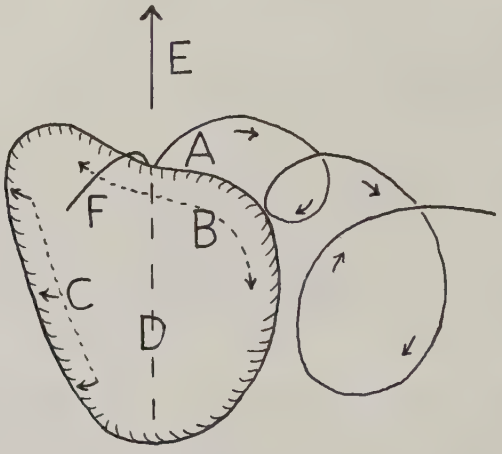


FIG. 22. "*Monas stigmatica* with its two flagella. Diagrammatic." "Waves pass along the long flagellum, from base to tip, in a spiral manner with an increase in velocity and amplitude. The force generated is transmitted to the surface of the cell at *A*. This causes the organism to rotate and gyrate about the axis *D*. (The edge *B* going below the surface of the paper and *C* being raised above it.) Thus the organism is converted into a rotating inclined plane and moves forward more or less in the direction indicated by the arrow *E*. The very short flagellum *F* appears to act as a guiding or sensory organ during normal swimming." (Redrawn and quoted from Lowndes, 1945 a.)

speed. At a rate of body gyration of only about 1/sec. the relative speed may be at least 5. I should think that the forward component produced by the flagellum would aid greatly in the attainment of high speed. But perhaps my imagination is not keyed to the physics of the micro-world of the flagellum. Apparently the mechanics and physical relationships (e. g., the relative viscosity of water, a major item in the protozoan world) to which we are accustomed are tremendously altered, practically non-existent, at this level of dimensions. For an interesting discussion of some of the differences see Bidder (1923, pp. 304–307).

Brief mention may be made of the possibility of studying living organisms with the electron microscope. It would obviously be desirable to study such a structure as the flagellum in the living, intact state. Several papers have appeared relative

to the possibility of observing living structures with the instrument, but, so far as I know, the only objects studied thus or even very practical for such study have been spores. Perhaps the most ambitious attempts have been made with the closed cell described by Abrams and McBain (1944). The obvious difficulties result from greatly reduced contrast and Brownian movement, which prevent photographing of the objects under observation. Another major factor is the killing effect (aside from heat production) due to " . . . the electrons bounding down through the specimen and breaking the chemical bonds of the specimen. Breaking the bonds is detrimental to all living organisms. Every bond in living organisms does not have to be intact, but the critical molecules in the living organisms must have a specific structure in the organism for it to grow and reproduce itself. When you consider that, in maximum intensity, the beam reaches a high value of 1,000 electrons per square angstrom per second, you can realize that it wouldn't take the scattering of many electrons to kill such an organism." (Anderson, 1943.) In terms more familiar to the biologist, this electron bombardment would amount to 100,000,000,000 electrons striking a surface of 1 square micron each second. The average protozoan body would present a surface of a considerable number of square microns. Thus the prospects of studying a living flagellum, even if it were perfectly stationary and met the other requirements, would not be encouraging.

Summary

1. The work of previous investigators in this field is reviewed and discussed.

2. The structure of the flagellum of several species of Protozoa has been investigated by means of the electron microscope. Micrographs are included which show that:

(a) The flagella studied are of approximately uniform diameter throughout their entire length.

(b) Each flagellum consists of a denser axial core (axoneme) and a less dense sheath surrounding the core.

(c) In the flagella of *Euglena* and *Astasia* the axial core appears to consist of two closely approximated fibers of equal size.

(d) The sheath appears to contain or to consist of a coiled fiber which encircles the axial core in a helix.

(e) The flagella of *Euglena* and *Astasia* bear, along one side, what appears to be a single row of delicate filaments (flimmer) extending from the sheath. These flimmer have an average length of about 5–6 times the diameter of the flagellum proper.

(f) The long flagellum of *Ochromonas* bears similar filaments along both (or all?) sides.

(g) The flagella of *Chilomonas* bear no such lateral filaments.

The significance of certain details present in the electron micrographs is discussed.

3. The presence of two equal fibers in the flagellum of *Euglena*, together with the fact (demonstrated by previous investigators) that the flagellum of this form has two separate roots, indicates the ancestral formation of its flagellum by the fusion or union of two flagella. The hypothesis of a biflagellate ancestry of *Euglena* is strengthened.

4. The possibility of the origin of the primitive protozoan flagellum through the union within a common matrix of several simple fibrillar flagella (as are present in certain bacteria) is suggested. Evidence for such a theory is presented.

5. Observations were made on the activity of living flagella. The results were in direct confirmation of Lowndes's contentions that:

(a) The flagellum beats in spiral undulations.

(b) The waves of contraction progress from the base toward the tip of the flagellum.

(c) As they progress, the waves often increase in amplitude.

(d) The flagellum usually serves to push, rather than to pull the organism through the water, although it arises from the anterior end of the body.

6. Experiments were performed by means of actual underwater swimming by the author and with an "artificial flagellate" to determine the forces produced by gyrating bodies in water. In both cases, a simple conical gyratory movement was found sufficient to produce locomotion. Employing the artificial flagellate, it was found that rotation and gyration of a small body at about 6 gyrations per second was capable of producing a relative speed of as much as 7.0 (i. e., 7 lengths per second), in spite of the fact that the body had to pull along after it an object much larger than itself. This confirms the theory of Lowndes that the rotation and gyration of the body alone may adequately account for the locomotion of many flagellates, without any forward component produced directly by the flagellum.

7. A hypothesis is suggested concerning the mechanism of flagellar function: Perhaps the helical fiber of the sheath transmits an impulse which stimulates local contraction in the underlying fibrils. Possible ways by which such a mechanism might account for the various types of flagellar movement are mentioned.

8. Promising techniques, recently developed in other fields, are suggested for further research on flagella.

LITERATURE CITED

Abrams, I. M., and J. W. McBain. 1944. A closed cell for electron microscopy. Science 100 (2595): 273–274, 1 fig.

Alexeieff, A. 1924. Comparaison entre la structure des spermatozoides et celle des flagellés. Arch. f. Protistenk, 49: 104–111, 2 figs.

Allen, E. A. 1936. A Pentatrichomonas associated with certain cases of enterohepatitis or "blackhead" of poultry. Trans. Amer. Micr. Soc. 55: 315–322, 1 pl.

Alverdes, F. 1922. Studien an Infusorien über Flimmerbewegung, Lokomotion, und Reizbeantwortung. Schaxel: Arbeiten aus dem Gebiet der exp. Biol., Heft 3. 127 pp., 46 figs.

Anderson, T. F. 1943. Year Book of The Electron Microscope Society of America. (Record of oral discussion, p. 34.)

Awerinzew, S. von. 1907. Beiträge zur Kenntnis der Flagellaten. Zool. Anz. 31 (25): 834–841, 9 figs.

Ballowitz, E. 1890. Fibrilläre Struktur und Contraktilität. Pflügers Arch. ges. Physiol. 46: 433–464.

1908. Über den feineren Bau der eigenartigen, aus drei freien dimorphen Fasern bestehenden Spermien der Turbellarien. Arch. f. mikr. Anat. 71: 4–21, 3 pls.

Bancroft, F. W. 1913. Heliotropism, differential sensibility, and galvanotropism in *Euglena*. Jour. Exp. Zool. 15: 383–428, 5 figs.

Barker, D. 1943. Recent work on flagellar movement. New Phytol. 42: 49–53.

Baylor, M. R. B., A. Nalbandov, and G. L. Clark. 1943. Electron microscope study of sperm. Proc. Soc. Exp. Biol. and Med. 54: 229–232, 7 figs.

Bidder, G. P. 1923. The relation of the form of a sponge to its currents. Quart. Jour. Micr. Sci., N.S. 67: 293–323, 12 figs.

Burton, E. F., and W. H. Kohl. 1942. The Electron Microscope. Reinhold Publishing Corp., New York.

Bütschli, O. 1883–1887. Protozoa: Mastigophora. Bronns Klassen und Ordnungen des Thier-reichs. Band 1, Abt. 2.

1902. Bemerkungen über Cyanophyceen und Bacteriaceen. Arch. f. Protistenk. 1: 41–58, 1 pl.

Calkins, G. N. 1933. The Biology of the Protozoa, 2nd ed. rev. Lea and Febiger, Philadelphia and New York.
Carter, G. S. 1924. On the structure and movements of the latero-frontal cilia of the gills of *Mytilus*. Proc. Roy. Soc. London 96 B: 115–122, 14 figs.
Claude, A., and E. F. Fullam. 1945. An electron microscope study of isolated mitochondria. Jour. Exp. Med. 81 (1): 52–62, 2 pls.
Dangeard, P. A. 1901 a. Étude sur la structure de la cellule. Le Botaniste, 8*e* Série, Poitiers: 5–58, 4 figs.
1901 b. Recherches sur les eugléniens. Ibid., 97–370, 4 pls., 53 figs.
Deflandre, G. 1923. Emploi de la Nigrosin dans l'étude des algues inférieures. Bull. Soc. bot. de France 70: 738–741.
1934. Sur la structure des flagelles. Anns. Protistol. 4: 31–54, 5 pls., 14 figs.
Delage, Y., et Hérouard. 1896. Traité de Zoologie Concrète, I. La Cellule et les Protozoaires. Schleicher Frères, Paris.
Dellinger, O. P. 1909. The cilium as a key to the structure of contractile protoplasm. Jour. Morph. 20: 171–210, 4 pls. (of photomicrographs), 13 figs.
Doflein, F. 1929. Lehrbuch der Protozoenkunde, 5th ed. (re-edited by E. Reichenow). Verlag von Gustav Fischer, Jena.
Duboscq, O., et P. Grassé. 1933. L'appareil parabasal des flagellés. Archs. Zool. Exp. et Gen. 73: 381–621, 4 pls. (With an extensive bibliography.)
Ellinger, P. 1940. Fluorescence microscopy in biology. Cambridge Philosoph. Soc., Biol. Rev. 15: 323–350. (Good bibliography.)
Emmel, L., A. Jakob, und H. Gölz. 1943. Elektronenoptische Untersuchungen an Malaria Sporozoiten und Beobachtungen an Kulturformen von *Leishmania donovani*. Deutsche tropenmed. Zeitschr. 46 (13): 354–358, 9 figs.
Engelmann, T. W. 1881. Über den faserigen Bau der contraktilen Substanzen, usw. Arch. ges Physiol. 25: 538–565, 1 pl.
Entz, G. 1926. Beiträge zur Kenntnis der Peridineen. I. Zur Morphologie und Biologie von *Peridinium Borgei* Lemmermann. Arch. f. Protistenk. 56: 397–446, 1 pl., 33 figs.
1928. Über den Bau und über die Tätigkeit der Geisseln der Peridineen. Anns. Protistol. 1: 75–95, 5 tables, 29 figs.
Erhard, H. 1910. Studien über Flimmerzellen. Arch. f. Zellforsch. 4: 309–442, 2 pls., 16 figs., 250 references in bibliography.
Fischer, A. 1894. Über die Geisseln einiger Flagellaten. Prings. Jahrb. f. wiss. Bot. 26: 187–235, 2 pls.
1895. Ibid., 27: 1–163, 5 pls.
Friedrich, L. 1909. Über Bau und Naturgeschichte des *Trypanoplasma helicis* Leidy. Arch. f. Protistenk. 14: 363–395, 48 figs.
Gelei, J. von. 1926. Zur Kenntnis des Wimperapparates. Zeitschr. f. Anat. u. Entwicklungsgeschichte 81: 530–553, 24 figs.
Gerloff, J. 1940. Beiträge zur Kenntnis der Variabilität und Systematik der Gattung *Chlamydomonas*. Arch. f. Protistenk. 94: 311–502, 48 figs.
Gicklhorn, J. 1921. Eine einfache Methode zur Herstellung der Geissel mit Basalkorn bei Flagellaten, besonders bei Eugleninen. Zeitschr. f. wiss. Mikr. 38: 123–129.
Goldschmidt, R. 1907. Lebensgeschichte der Mastigamöben usw. Arch. f. Protistenk., Supplement I: 83–168, 5 pls., 20 figs.
Grassé, P. P. 1926. Contribution a l'étude des flagelles parasites. Archs. Zool. Exp. et Gen. 65: 345–602, 12 pls., 76 figs.
Gray, J. 1922. The mechanism of ciliary movement. Proc. Roy. Soc. London 93 B: 104–121, 7 figs.
1928. Ciliary Movement. 162 pp., 105 figs., 16 tables. Cambridge Univ. Press, London. (Rather complete bibliography.)
Günther, F. 1928. Über den Bau und die Lebensweise der Euglenen. Arch. f. Protistenk. 60: 511–590, 3 pls., 5 figs.
***Gurwitsch, A.** 1904. Morphologie und Biologie der Zelle. Jena.
Hall, R. P., and T. L. Jahn. 1929. On the comparative cytology of certain euglenoid flagellates and the systematic position of the families Euglenidae Stein and Astasiidae Bütschli. Trans. Amer. Micr. Soc. 48: 388–405, 2 pls., 2 figs.
Hamburger, C. 1911. Studien über *Euglena Ehrenbergii*, insbesondere über die Körperhulle. Sitz.-ber. d. Akad. d. Wiss., Heidelberg. Abhandlung 4.
Hartmann, M., and C. Chagas. 1910. Flagellaten-Studien. Mem. Inst. Oswaldo Cruz 2: 64–125, 6 pls., figs.
Harvey, E. B., and T. F. Anderson. 1943. The spermatozoon and fertilization membrane of *Arbacia punctulata* as shown by the electron microscope. Biol. Bull. 85 (2): 151–156, 2 pls.
Harvey, E. N., and A. L. Loomis. 1931. High speed photomicrography of living cells subjected to supersonic vibrations. Contrib. Biol. Labs., Princeton Univ., 10 (21). Reprinted from Jour. Gen. Physiol. 15 (2): 147–153, 3 figs.

Hawley, G. G. 1945. Seeing the Invisible. The story of the Electron Microscope. A. A. Knopf, New York.
Heidenhain, M. 1911. Plasma and Zelle II. Fischer, Jena.
Hillier, J., and A. W. Vance. 1941. Recent developments in the electron microscope. Proc. Inst. Radio Eng., Inc. 29: 167–176, 14 figs.
Hutchinson, W. G., and M. R. McCracken. 1943. Study of flagella of a freshwater bacterium by motion microphotography and electron micrography. (Abstract) Jour. Bact. 45: 305.
Hyman, L. H. 1940. The Invertebrates: Protozoa through Ctenophora. McGraw-Hill, New York.
Kater, J. McA. 1929. Morphology and division of *Chlamydomonas* with reference to the phylogeny of the flagellate neuromotor system. Univ. Calif. Publ. in Zool. 33: 125–168, 6 pls., 7 figs.
Keysselitz, G. 1906. Generations- und Wirts-wechsel von *Trypanoplasma borreli* Laveran et Mesnil. Arch. f. Protistenk. 7: 1–74, 162 figs.
Kirby, H. 1943. Observations on a trichomonad from the intestine of man. Jour. Parasitol. 29 (6): 422–423.
Klebs, G. 1892. Flagellatenstudien I, II. Zeitschr. f. wiss. Zool. 55: 265–445, 6 pls., 122 references.
Klein, B. M. 1929. Weitere Beiträge zur Kenntnis des Silberliniensystems der Ciliaten. Arch. f. Protistenk. 65: 183–257, 1 pl., 45 figs.
Kofoid, C. A., and O. Swezy. 1919. Studies on the parasites of termites. III. On *Trichonympha campanula*, sp. nov. Univ. Calif. Publ. in Zool. 20: 41–98, 11 pls., 4 figs.
1920. On the morphology and mitosis of *Chilomastix mesnili* (Wenyon), a common flagellate of the human intestine. Ibid., 117–144, 5 pls., 2 figs.
1923. On the morphology and behavior of *Pentatrichomonas ardin delteili* (Derrieu et Raynaud). Ibid., 373–390, 1 pl., 1 fig.
Koltzoff, N. K. 1903. Über formbestimmende elastische Gebilde in Zellen. Biol. Centralbl. 23: 680–696, 12 figs.
1909. Studien über die Gestalt der Zelle. II. Untersuchungen über das Kopfskelett des tierischen Spermiums. Arch. f. Zellforsch. 2: 1–65, 5 pls., 18 figs.
Korschikov, A. 1923. Über den Bau und die Aggregation der Geisseln bei den Volvocales und den Flagellaten. Archs. Soc. Russe de Protistol. 2: 195–205, 4 figs.
Krijgsman, B. J. 1925. Beiträge zum Problem der Geisselbewegung. Arch. f. Protistenk. 52: 478–488, 6 figs.
Kudo, R. R. 1939. Protozoology, 2nd ed. C. C. Thomas, Springfield, Ill.
***Kunstler, J.** 1882. Contribution à l'étude des flagellés. Bull. Soc. Zool. de France 7: 20.
1889. Recherches sur la morphologie des flagellés. Bull. Sci. de la France et de la Belgique 20: 399–515, 9 pls.
Laveran, A., and F. Mesnil. 1907. Trypanosomes and Trypanosomiases. (Translation by D. Nabarro.) W. T. Keener and Co., Chicago.
Lavin, G. I., and C. L. Hoagland. 1943. Ultraviolet photomicrography of muscle. Proc. Soc. Exp. Biol. and Med. 52: 80–82, 14 figs.
Loeffler, F. 1889. Eine neue Methode zum Färben der Mikroorganismen, im besonderen ihrer Wimperhaare und Geisseln. Centralbl. f. Bakt. 6: 209–224, 2 pls.
1890. Weitere Untersuchungen über die Beizung und Färbung der Geisseln bei den Bakterien. Ibid., 7: 625.
Lowndes, A. G. 1935. The twin polygraph and strobograph. Nature 135: 1006–1007, 1 photograph of assembled apparatus.
1936. Flagella movement. Ibid., 138: 210–211, 4 figs.
1941 a. Mechanics of a flagellum. Ibid., 148: 198, 5 figs.
1941 b. On flagellar movement in unicellular organisms. Proc. Zool. Soc. London 111 A: 111–134, 20 figs.
1943. The term *tractellum* in flagellate organisms. Nature 152: 51.
1944 a. The swimming of unicellular flagellate organisms. Proc. Zool. Soc. London 113 A: 99–107, 6 figs.
1944 b. The swimming of *Monas stigmatica* Pringsheim and *Peranema trichophorum* (Ehrbg.) Stein and *Volvox* sp. Additional experiments on the working of a flagellum. Ibid., III, 114: 325–338, 10 figs.
1945 a. Swimming of *Monas stigmatica*. Nature 155: 579, 1 fig.
1945 b. The swimming of Euglena and flagellar movement in general. The School Science Review, No. 100, June, 1945. pp. 319–332, 2 pls., 9 figs.
Lucas, F. F. 1930. The architecture of living cells.—Recent advances in methods of biological research.—Optical sectioning with the ultra violet microscope. Tech. Publ. Bell Telephone System, Monograph B 514, 9 pp., 16 figs. (Also in Proc. Natl. Acad. Sci. 16: 599–607.
1934. Late developments in microscopy. Ibid., Monograph B-792, 47 pp., 32 figs.

Mainx, F. 1928. Beiträge zur Morphologie und Physiologie der Eugleninen. Arch. f. Protistenk. 60: 305–414, 1 plate containing 18 photomicrographs, 8 figs. (Good bibliography.)

Marsland, D. A. 1943. Quieting *Paramecium* for the elementary student. Science 98 (2549): 414.

Marton, L. 1941. The electron microscope. A new tool for bacteriological research. Jour. Bact. 41: 397–413, 12 figs.

1943. The electron microscope in biology. Ann. Rev. Biochem. 12: 587–614. Annual Reviews Inc., Stanford University.

McClung, C. E. (editor). 1937. Handbook of microscopical technique. Paul B. Hoeber, Inc., Medical Book Dept. of Harpers and Brothers, New York.

McDonald, J. D. 1922. On *Balantidium coli* (Malmsten) and *Balantidium suis* n. sp., with an account of their neuromotor apparatus. Univ. Calif. Publ. in Zool. 20: 243–300, 2 pls., 15 figs.

Metcalf, R. L., and R. L. Patton. 1944. Fluorescence microscopy applied to entomology and allied fields. Stain Technol. 19 (1): 11–27, 1 pl., 1 fig.

Metzner, P. 1920. Zur Mechanik der Geisselbewegung. Biol. Centralbl. 40 (2): 49–87, 18 figs., 48 references.

Minchin, E. A. 1922. An Introduction to the Study of the Protozoa. Edward Arnold, London.

Morton, H. E., and T. F. Anderson. 1942. Some morphological features of the Nichols strain of *Treponema pallidum* as revealed by the electron microscope. Amer. Jour. Syphilis, Gonorrhea, and Venereal Dis. 26 (5): 565–573, figs.

Mudd, S., and T. F. Anderson. 1942. Selective "staining" for electron micrography. The effects of heavy metal salts on individual bacterial cells. Jour. Exp. Med. 76 (1): 103–108, 4 pls.

1944. Pathogenic bacteria, rickettsias, and viruses as shown by the electron microscope. Amer. Med. Assn., Chicago. (Reprinted, with additions, from the Jour. Amer. Med. Assn. 126: 561–571, 632–639.) 24 pp., 50 figs.

Mudd, S., K. Polevitsky, T. F. Anderson, and L. A. Chambers. 1941. Bacterial morphology as shown by the electron microscope. II. The bacterial cell-wall in the genus Bacillus. Jour. Bact. 42: 251–264, figs.

Mudd, S., K. Polevitsky, and T. F. Anderson. 1943. Bacterial morphology as shown by the electron microscope. V. *Treponema pallidum*, *T. macrodentium*, and *T. microdentium*. Jour. Bact. 46: 15–24, 8 figs.

Nigrelli, R. 1929. On the cytology and life-history of *Trypanosoma diemyctyli* and the polymorphonuclear count of infected newts (*Triturus viridescens*). Trans. Amer. Micr. Soc. 48: 366–387, 2 pls., 1 fig., 1 table.

Petersen, J. B. 1918. Om *Synura uvella* Stein og nogle andre Chrysomonadiner. Vidensk. Medd. fra Dansk naturhist. Foren 69: 345–357, 1 pl.

1929. Beiträge zur Kenntnis der Flagellatengeisseln. Botanisk Tidsskrift (Copenhagen) 40 (5): 373–389, 1 pl.

Petrová, J. 1931. Die vermeintliche Heterokonte *Botrydiopsis minor*—eine Chlorophycee. Beihefte z. bot. Centralbl. 48: 221–228, 7 figs.

Piekarski, G., und H. Ruska. 1939. Übermikroskopische Darstellung von Bakteriengeisseln. Klin. Wochenschr. 18 (1): 383–386, 11 figs.

Pijper, A. 1940. Microcinematography of the motile organs of the typhoid bacillus. Jour. Biol. Photog. Assn. 8: 158–164, figs.

1941. Microcinematography of the agglutination of typhoid bacilli. Jour. Bact. 42: 395–409, figs.

Plehn, M. 1904. *Trypanoplasma cyprini* n. sp. Arch. f. Protistenk. 3: 175–180, 1 pl.

***Plenge, H.** 1898. Über die Verbindung zwischen Geissel und Kern bei den Schwärmzellen der Mycetozoen und bei Flagellaten. Verh. nat. Ver., Heidelberg, N.F. 6.

Polevitsky, K. 1941. Pictures of bacterial forms taken with the electron microscope. (Abs.) Jour. Bact. 41: 260.

Porter, K. R., A. Claude, and E. F. Fullam. 1945. A study of tissue culture cells by electron microscopy. Jour. Exp. Med. 81 (3): 233–246, 5 pls.

Prebus, A. F. 1944. The electron microscope. Colloid Chemistry V (Edited by Jerome Alexander). Reinhold Publ. Co., New York, pp. 152–235, figs.

Prowazek, S. von. 1903. Flagellatenstudien. Arch. f. Protistenk. 2: 195–212, 2 pls.

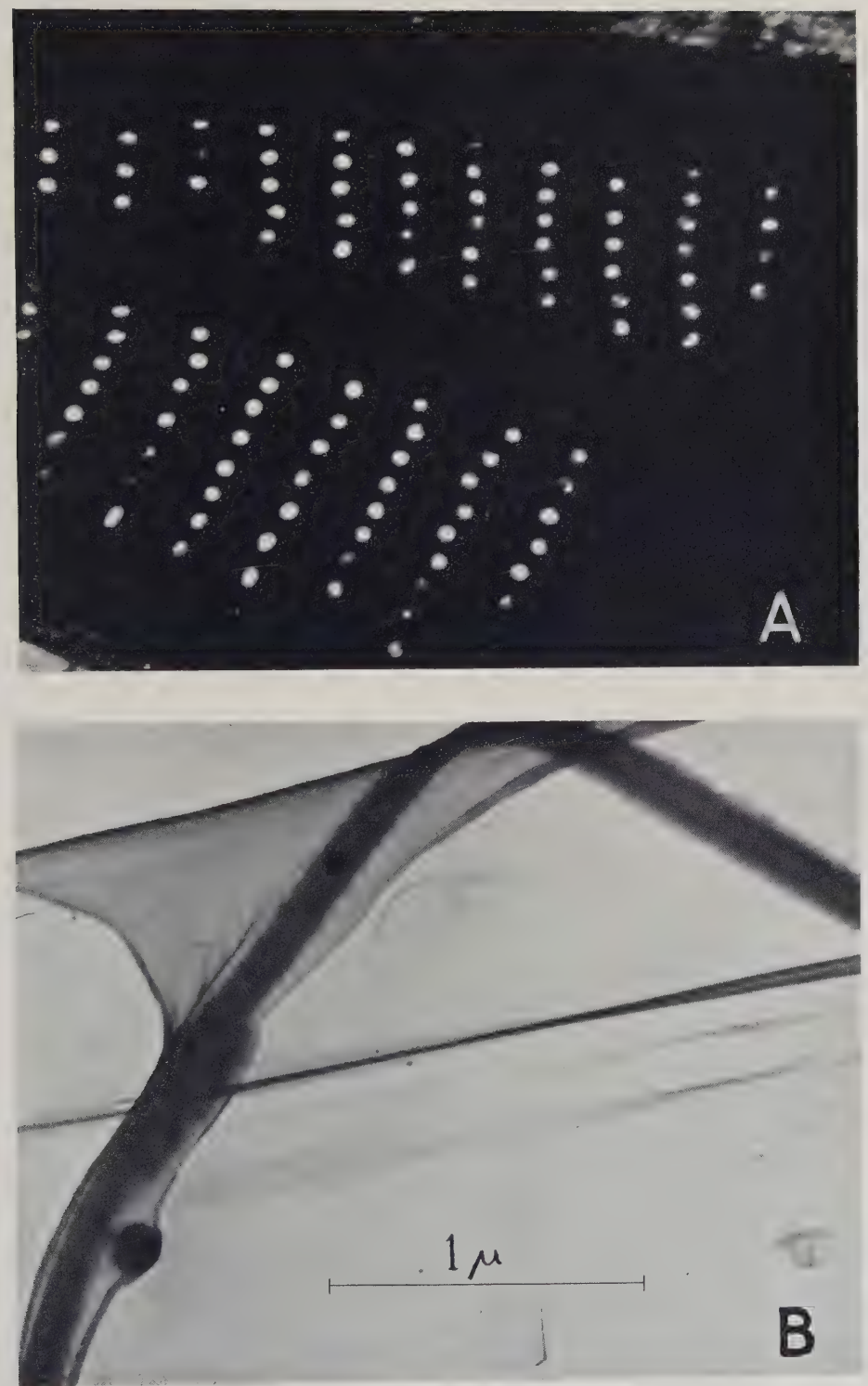
Pütter, A. 1903. Die Flimmerbewegung. Ergebnisse d. Physiol. 2 (2): 1–102, 15 figs., 208 references.

Reichert, K. 1909. Über die Sichtbarmachung der Geisseln und die Geisselbewegung der Bakterien. Centralbl. Bakt. 51: 14–94, 30 figs.

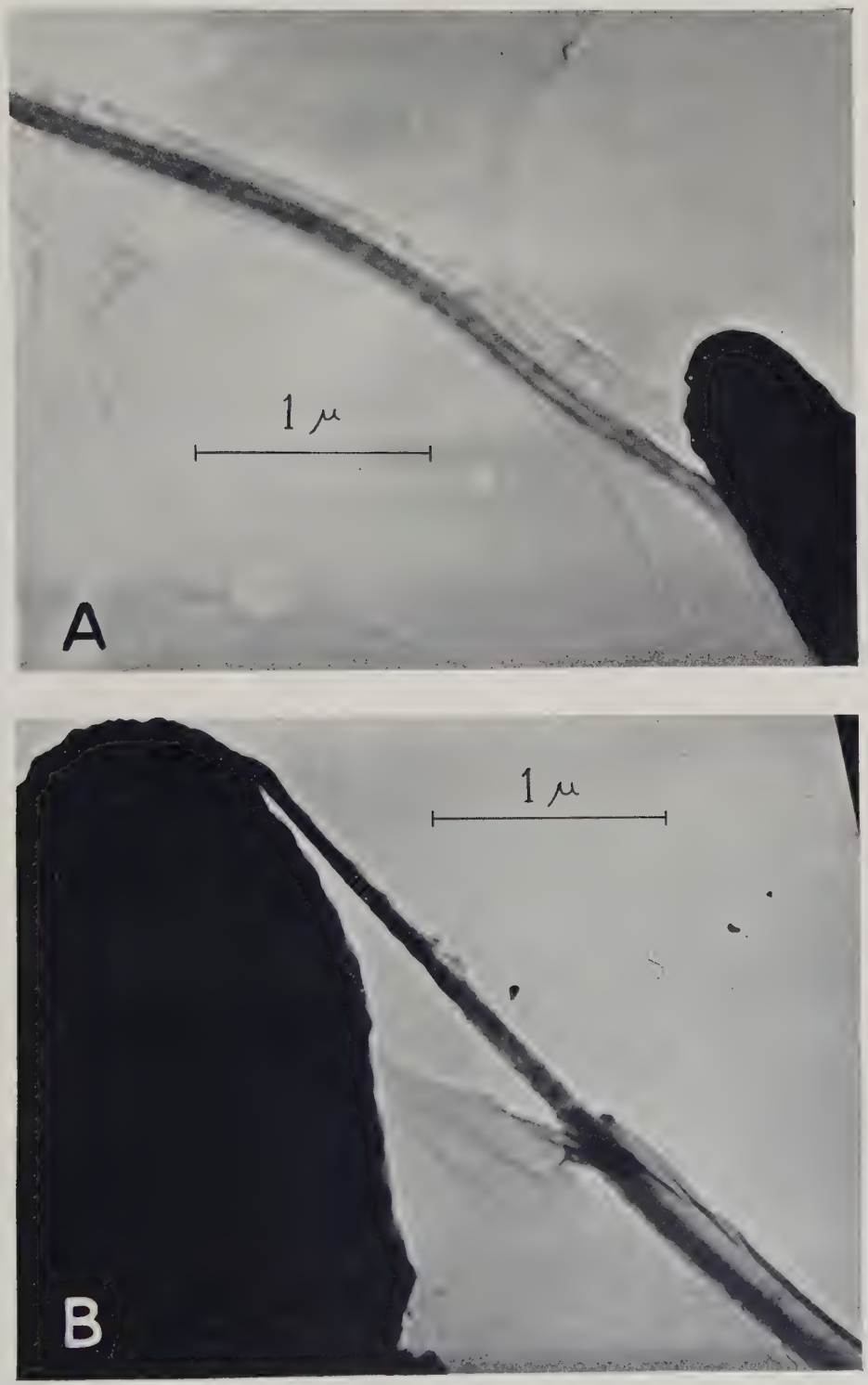
Richards, O. W. 1944. Phase difference microscopy. Nature 154: 672, 1 fig.

Richards, A. G., and T. F. Anderson. 1942. Electron microscope studies of insect cuticle, with a discussion of the application of electron optics to this problem. Jour. Morph. 71: 135–183, figs.

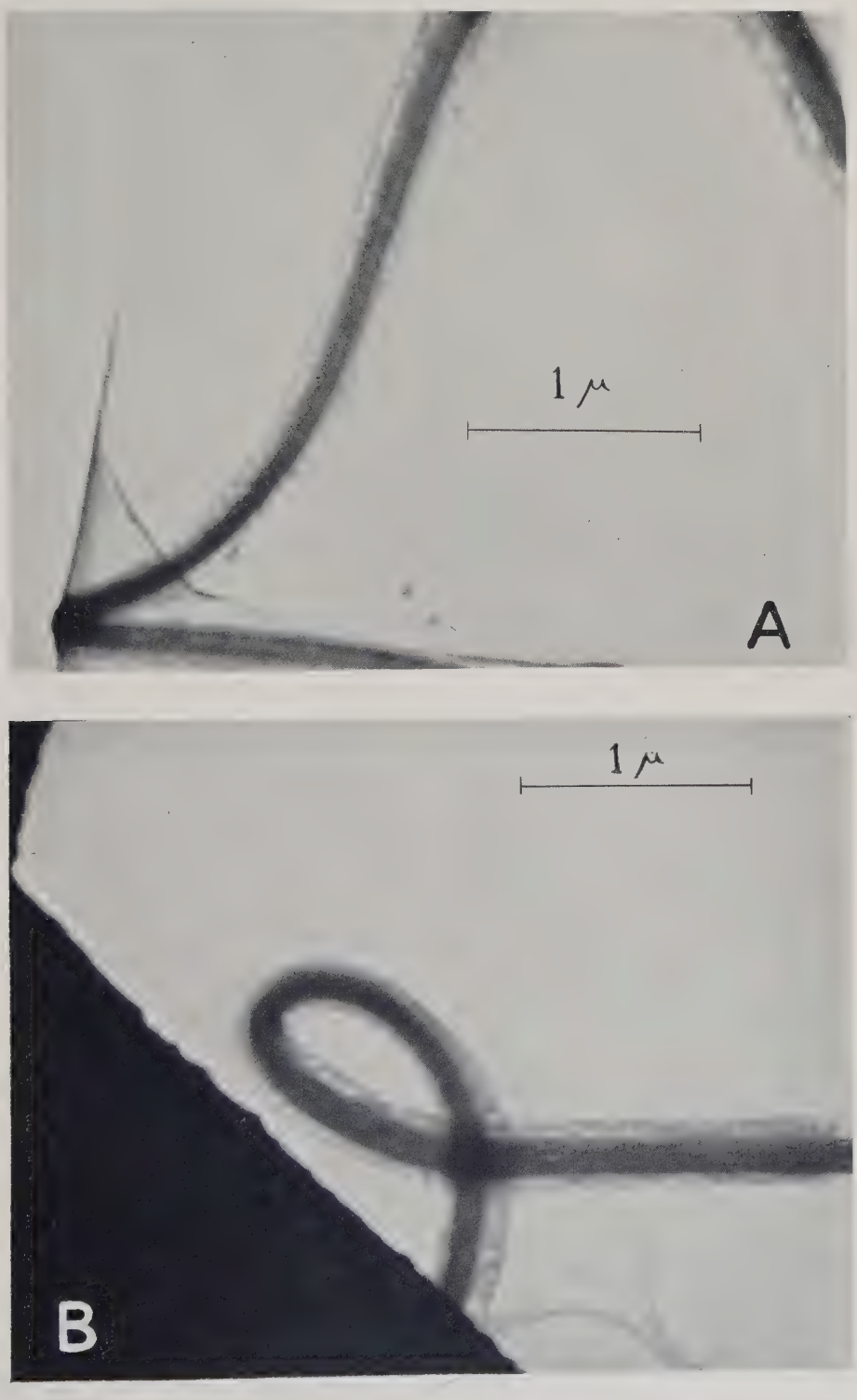
Rosenbusch, T. 1908. Trypanosomen-Studien. Arch. f. Protistenk. 15: 263–296, 3 pls.
Roskin, G. 1922. Über den Bau von kontraktilen Elementen und Stützsubstanzen einigen Protozoen. Archs. Soc. Russe de Protistol. 1: 35–45, 1 pl., 1 fig.
1923. Zur Frage über den Bau der Geisseln. Ibid. 2: 206–209, 3 figs.
1925. Über die Axopodien der Heliozoa und die Greiftentakeln der Ephelotidae. Arch. f. Protistenk. 52: 207–216, 9 figs.
Schindera, M. 1922. Beiträge zur Biologie, Agglomeration, und Züchtung von *Trypanoplasma helicis* Leidy. Arch. f. Protistenk. 45: 200–240, 1 pl., 3 figs.
Schmitt, F. O. 1939. The ultrastructure of protoplasmic constituents. Physiol. Rev. 19 (2): 270–302, 1 fig.
1944. Structural proteins of cells and tissues. Advances in Protein Chem. 1: 25–68, 9 figs.
Schmitt, F. O., C. E. Hall, and M. A. Jakus. 1943. The ultrastructure of protoplasmic fibrils. Frontiers in Cytochemistry, Biological Symposia X. Jaques Cattell Press, Lancaster, Pa. pp. 261–276, 2 pls.
Schouteden, H. 1907. Notes sur quelques flagellés. Arch. f. Protistenk. 9: 108–136, 11 figs.
Schuberg A. 1905. Über Cilien und Trichocysten einiger Infusorien. Arch. f. Protistenk. 6: 61–110, 2 pls.
Smyth, J. D. 1944. A technique for mounting free-living protozoa. Science 100 (2586): 62.
Ulehla, V. 1911. Ultramikroskopische Studien über Geisselbewegung. Biol. Centralbl. 31: 645–654, 657–676, 689–705, 721–731, 37 figs.
Verworn, M. 1890. Studien zur Physiologie der Flimmerbewegung. Arch. f. ges. Physiol. 48: 149–180, 4 figs.
1915. Allgemeine Physiologie, 6th ed. Fischer, Jena.
Vlk, W. 1931. Über die Struktur der Heterokontengeisseln. Beihefte z. bot. Centralbl. 48: 214–220, 15 figs.
1938. Über den Bau der Geissel. Arch. f. Protistenk, 90: 448–488, 1 pl., 12 figs. (Good bibliography and historical review.)
Wager, H. 1899. On the eyespot and flagellum in *Euglena viridis*. Jour. Linn. Soc. London, Zoology 27: 463–481, 1 pl.
Williams, L. W. 1907. The structure of cilia, especially in gastropods. The Amer. Naturalist 41 (489): 545–551, 2 figs.
Williams, R. C., and R. W. G. Wyckoff. 1944. The thickness of electron microscope objects. Jour. Appl. Phys. 15 (10): 712–715, 8 figs.
1945 a. Electron shadow-micrography of virus particles. Proc. Soc. Exp. Biol. and Med. 58 (3): 265–270, 6 figs.
1945 b. Electron shadow-micrography of the tobacco mosaic virus protein. Science 101 (2632): 594–596, 3 figs.



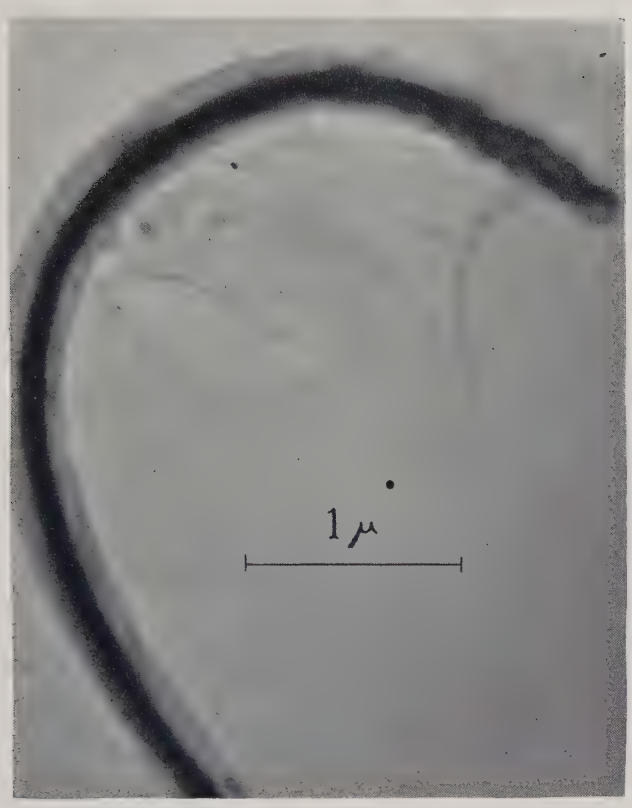
A. Portion of a diatom used for measurements and estimation of magnification. EM 1703 D. B. *Astasia klebsii*. Portion of flagellum showing unilateral flimmer and indications of helix of sheath as in Plate 9. EM 1712 D. Technique #1.

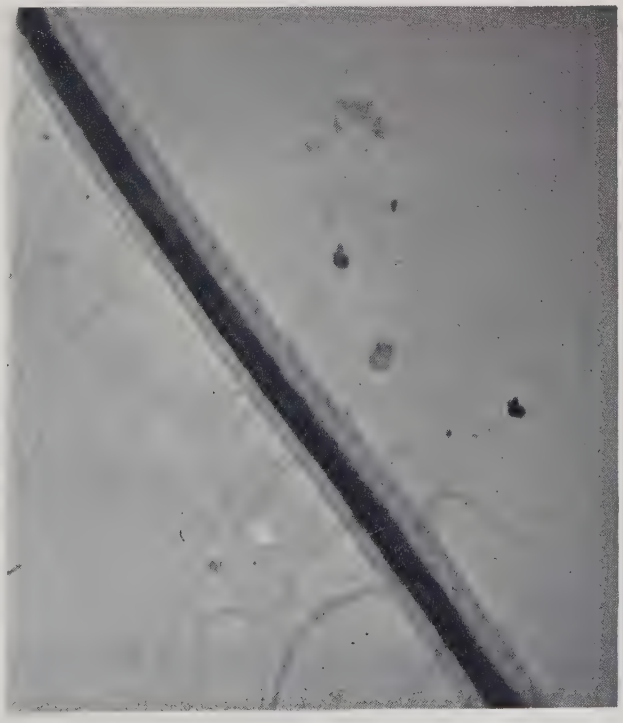


Euglena gracilis. Extremities of bodies and portions of flagella. Fig. A, EM 1704 D. Fig. B, EM 1696 D. Prepared according to technique #1.

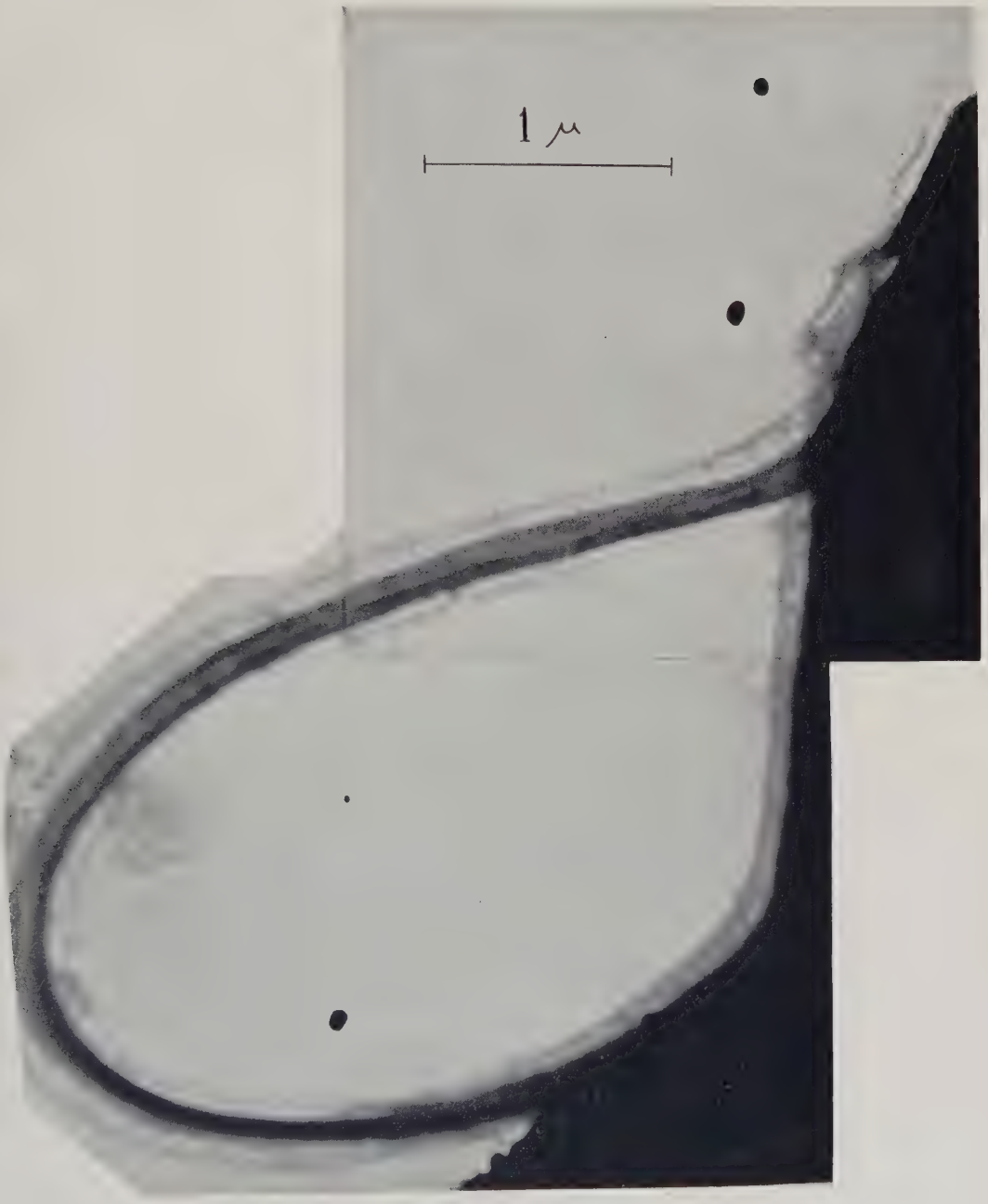


Euglena gracilis. Portions of flagella. In Fig. A (EM 1691 D), the helical nature of the sheath is apparent. Flimmer may be seen in Fig. B (EM 1687 D). Technique #1.

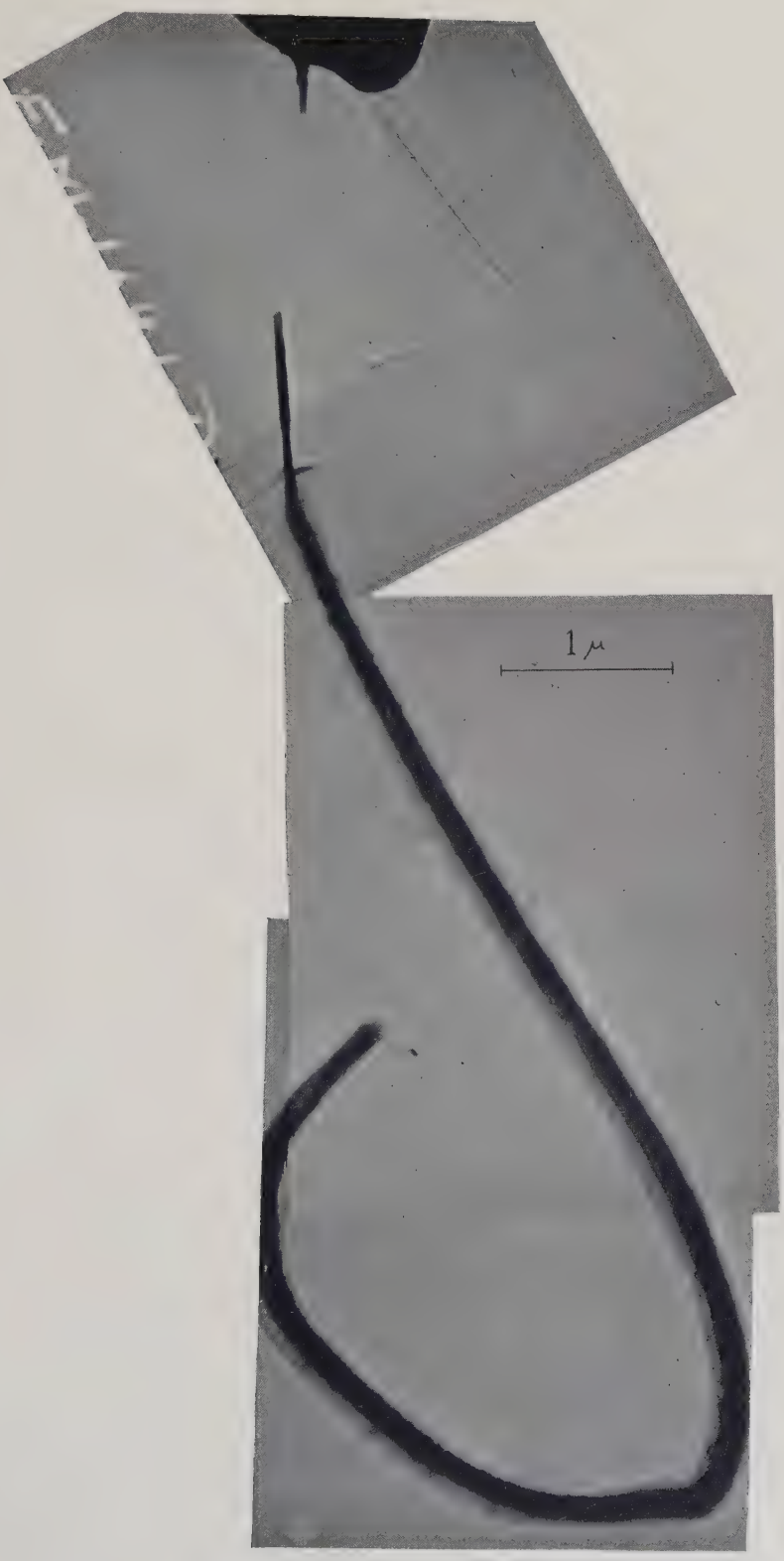




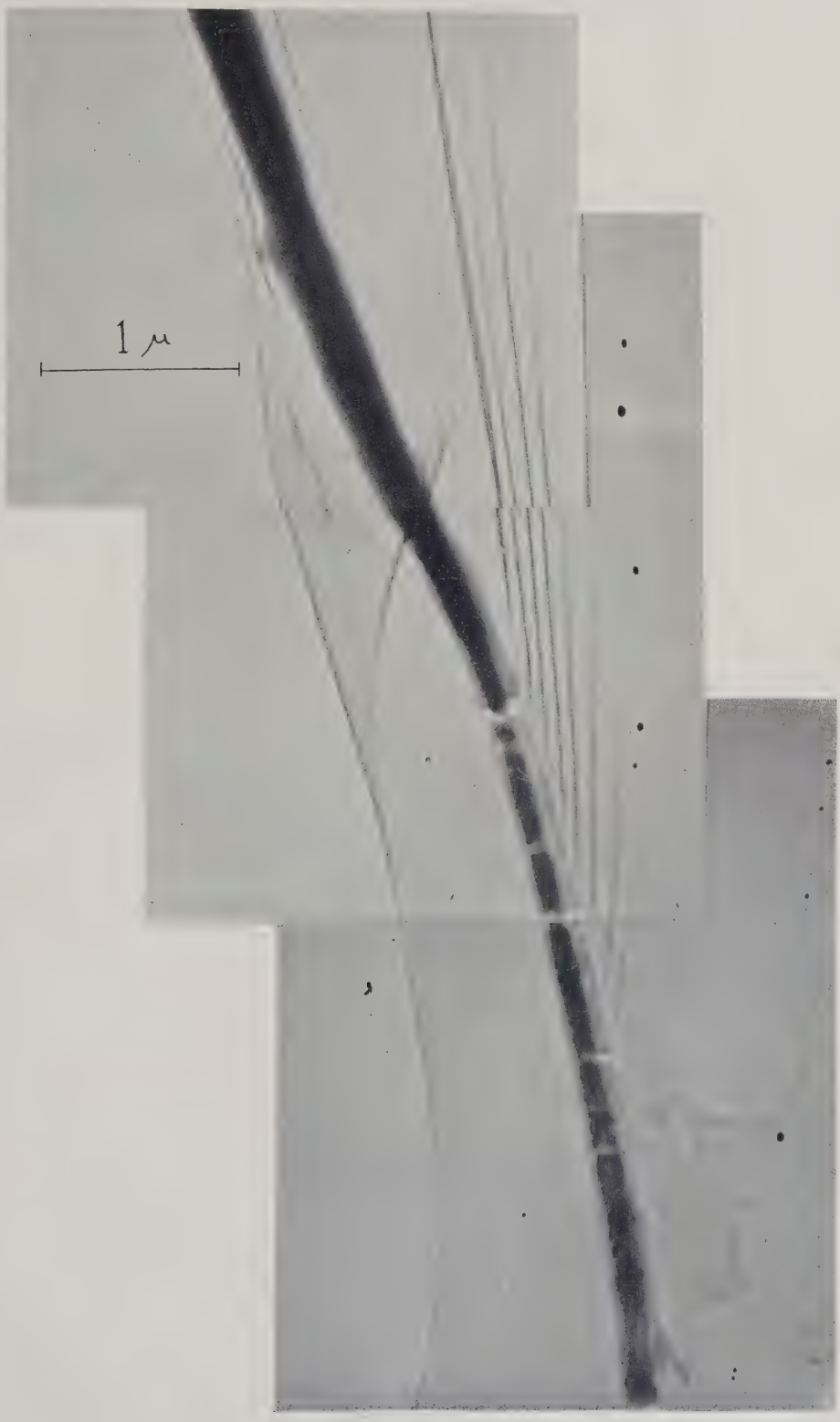
Euglena gracilis. Part of a flagellum showing unilateral flimmer especially well. EM 1700, 1701 D. Technique #1.



Euglena gracilis. The flagellum extends from the body, loops, and returns to lie alongside the body. EM 1710, 1711 D. Technique #1.

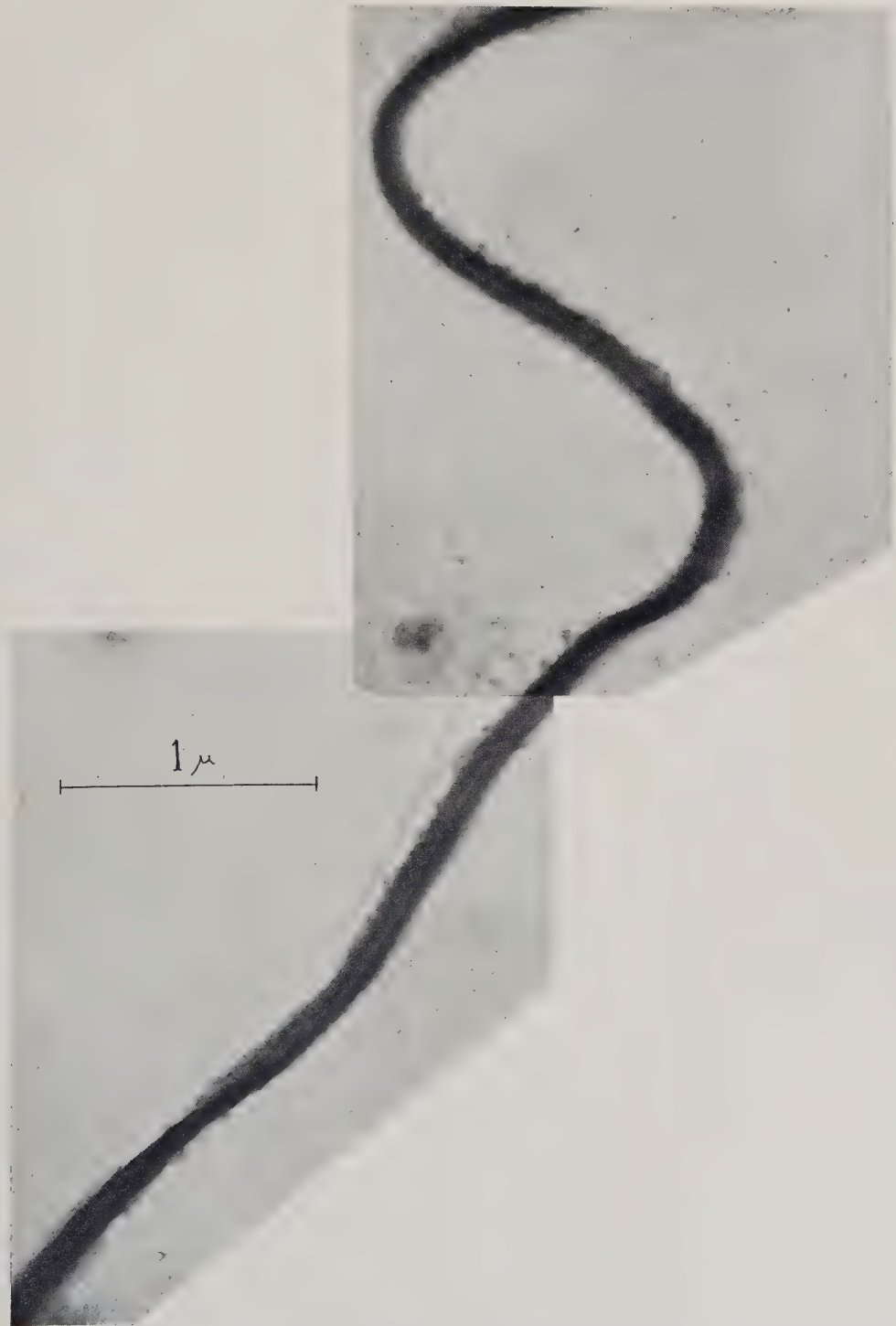


Euglena gracilis. In the upper section, the flagellum is seen to emerge from the body. It has been snapped by a rupture of the supporting film, and parts of the axoneme are exposed. The length of the flagellum is about 13 μ. EM 1697, 1698, 1699 D. Technique #1.

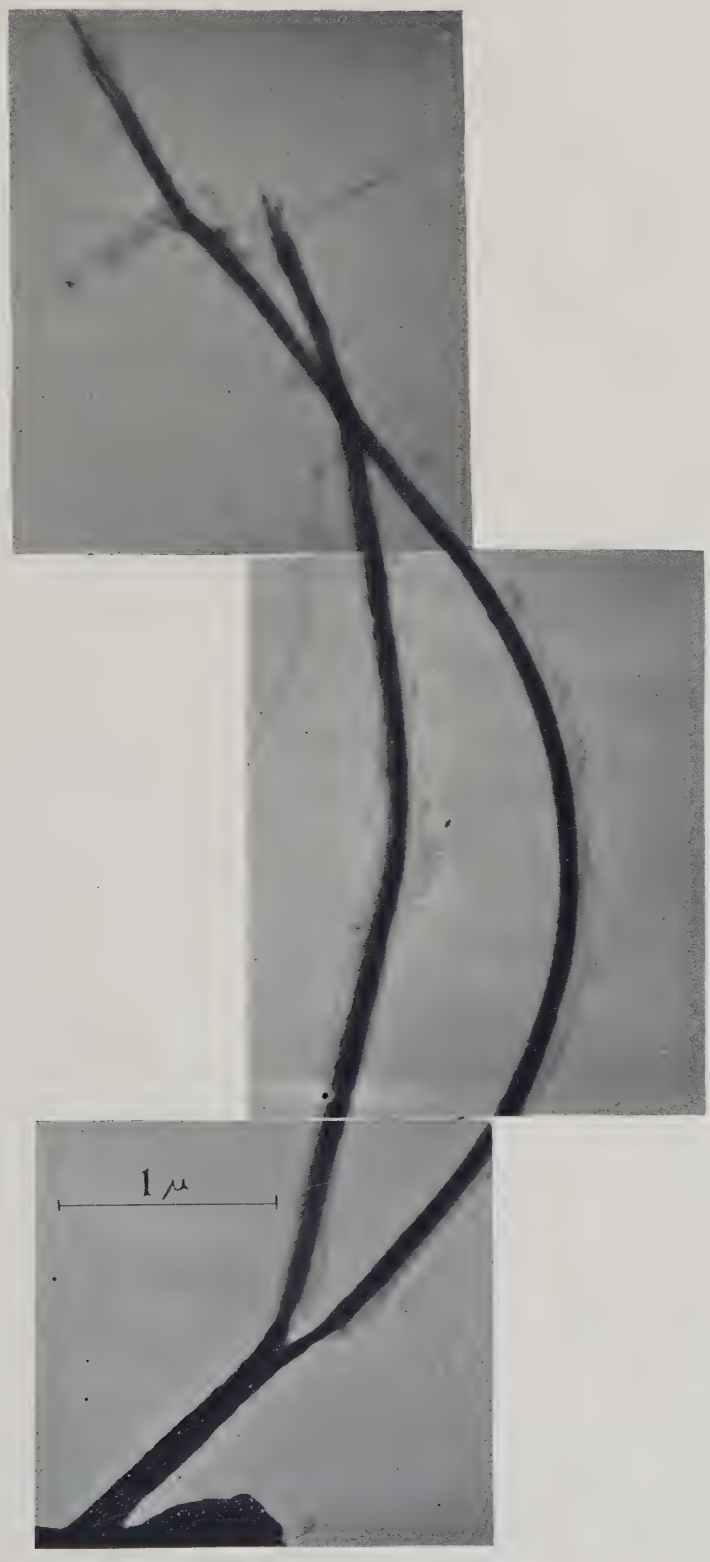


Euglena gracilis. Portion of the flagellum showing two very different appearances. The upper part perhaps represents the more nearly natural condition. See discussion in text. Note the transversely striated appearance of the upper, denser portion, produced by the closely wrapped helical fiber. EM 1692, 1693, 1694 D. Technique #1.

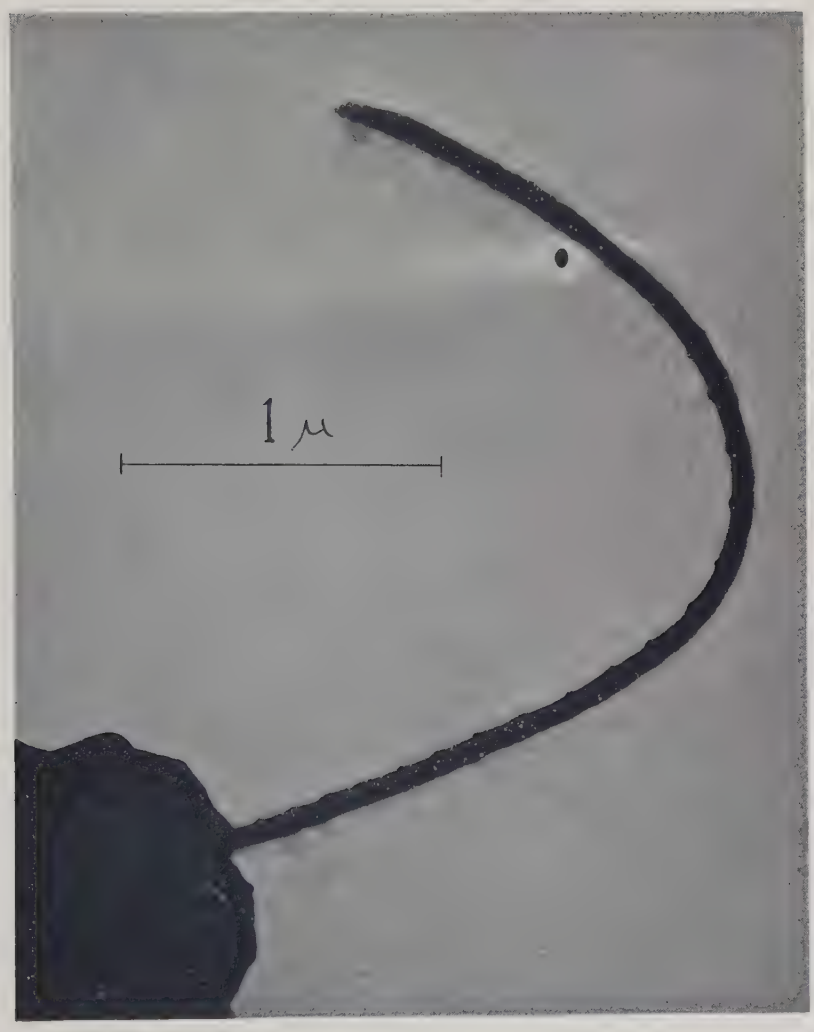
Euglena gracilis. Portion of flagellum showing a swollen appearance, perhaps due to the escape of flagellar plasm during drying. EM 1707, 1708 D. Technique #1.



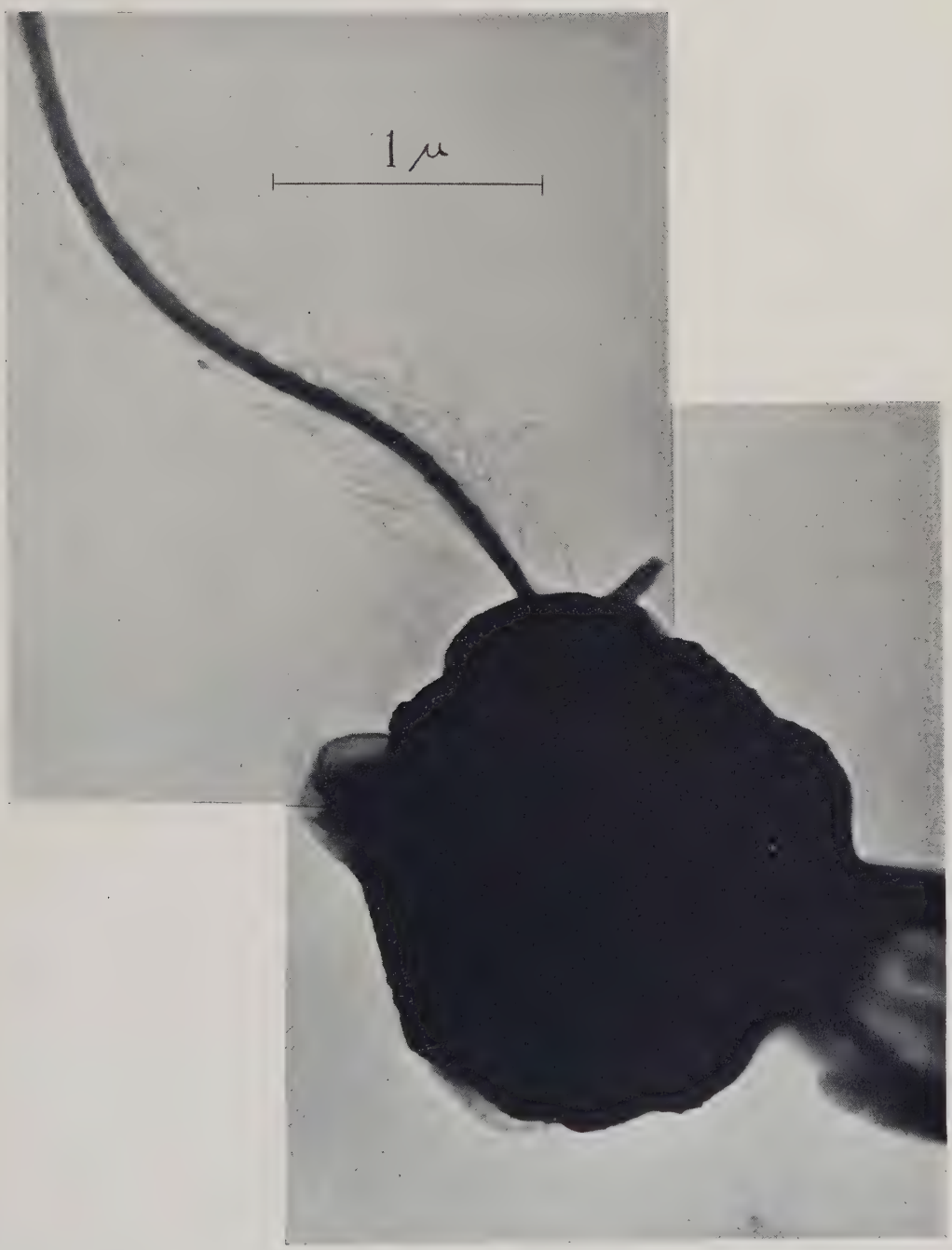
Euglena gracilis. Portion of flagellum showing unilateral flimmer. In places where the flagellum is twisted, the two equal fibers of the axoneme are made rather obvious. The helix of the sheath appears to be closely appressed to the axoneme (note regular bumps along sides of flagellum. EM 1620, 1621 D. Technique #2.



Chilomonas paramecium. Anterior end of body and the two flagella. No flimmer are to be observed. EM 1723, 1724, 1725 D. Technique #1.



Ochromonas variabilis. Portion of body and flagellum showing flimmer on both (all ?) sides, not in a single lateral row as on the flagellum of *Euglena.* The dimensions of *Ochromonas* flagella vary with the size of the organism, but in those photographed they ranged for the most part between 4 and 6.5 μ in length, averaging about 0.1 μ in diameter. The flimmer seem to average about 0.5 μ in length and are less than 0.01 μ in diameter. Note apparent helix as in Plates 9 and 1 B. EM 1745 D. Technique #1.



Ochromonas variabilis. The entire body of the organism is shown, with most of the flagellum. The small second flagellum may be seen near the base of the long flagellum. No flimmer have been observed upon it. Apparent helix as in Plate 11. EM 1737, 1739 D. Technique #1.

THE EYE-BANK FOR SIGHT RESTORATION

More than half a hundred hospitals in nine states are already cooperating with The Eye-Bank for Sight Restoration, Inc., in a nation-wide effort to help restore or remedy the vision of America's estimated 15,000 persons blinded because of corneal affections, it was announced recently by Mrs. Henry Breckenridge, executive director of the Eye Bank, 210 East 64th Street, New York.

In Greater New York alone, 32 hospitals are associated in the movement to make available for distribution healthy corneal tissue for those whose sight may be restored through corneal graft operations by which ocular opacity is overcome, the announcement stated.

In addition, 8 hospitals in other New York state cities, together with 6 in New Jersey, 3 in Connecticut and one each in six other states have become actively affiliated with the movement.

Organized only last February, the Eye Bank was established for the collection, preservation and distribution of healthy corneas which may be obtained only from persons either living or immediately after death. Inasmuch as corneas may be preserved and utilized for transplanting to the eyes of others for only 72 hours, speedy collection and distribution is essential as soon as they are obtained. Whenever cooperating hospitals have eyes available, the Red Cross Motor Corps rushes them to the Eye Bank for distribution to persons requiring the corneal graft operation.

In addition to extending this activity to hospitals throughout the United States, the Eye Bank is presently engaged in a nation-wide effort to obtain support for its work through solicitations for memberships and donations of eyes after death.

BOOK NOTICES

Indiana Climate

Isaiah Bowman in an address at one of the recent meetings has declared that "facts more valuable than all the gold of the Klondike lie hidden in the climatological records of the Weather Bureau." Whether or not it might be further remarked that an equally valuable set of climatic facts are overlooked or obscured by Weather Bureau methods of taking and recording data, does not detract at all from the vast store of climatic data Professor Stephen S. Visher has compiled and summarized from Weather Bureau records in a book titled "The Climate of Indiana."

That Professor Visher has sought out so much of the hidden value in the records is obvious at a glance. That he recognizes certain limitations of Weather Bureau records is clear through study of his work. The 27-chapter book contains 70 tables and almost 500 figures, 400 of which are maps of Indiana which emphasize various aspects of the state's climate. Moreover, these are not the conventional presentations of averages and extremes, but presentations of data that may be utilized by workers in biology, geography and other fields. That it is not simply calculation for calculation's sake may be judged from the following types of data which he has mapped: Days per decade during which temperature was continuously below 10°; percentages of Junes, Julys, Augusts which have had less than 1½″ of rain; times per 40 years that the crop-growing season was humid, moist sub-humid, dry; January, February, March, etc., 2-day rains of 2 inches or more per decade; normal date of beginning of corn planting; average number of days per year with maximum temperatures below 32°. These are only a few samples of many.

The thoroughness of Professor Visher's research cannot be told in little space but is suggested by the chapter titles. Chapter 1 is a general discussion of Indiana climate, and more or less of a summary of the next 15 chapters. These deal specifically and in meticulous detail with weather phenomena such as temperature—average, high and low; killing frosts; precipitation—normal and near normal, drouths, especially wet months and seasons, snowfall, rainfall intensity, hail and floods; humidity; sunshine and clouds; winds; thunderstorms, lightning, tornadoes. Contrasts of seasonal weather and seasonal weather types are discussed in two chapters.

In Chapters 19 to 24, climate is discussed with relation to physiography, crops, and health. One chapter is devoted to classifying Indiana climate. He recognizes 3 general divisions, the western, central and eastern thirds, each section being further subdivided into 3 or 4 divisions

north and south. One chapter is devoted to the climate of Bloomington and the final one is given over to comparative climatic data from 6 widely scattered stations.

The effects of climate on Indiana topography is an interesting summary, for climatic elements—wind, water and ice—have all played significant roles in the development of Indiana scenery. That the author is cognizant of the fact that the Weather Bureau methods and compilations obscure certain vital extremes in local areas is shown in his discussion of the effects of physiography on climate, although the treatment is brief and data fragmentary. His analysis of factors affecting crop plants is much more thorough and certainly more functional than most statements made by ecologists and weather bureau analysts themselves.

Professor Visher has obtained most of what is obtainable from Weather Bureau records for a region the size of Indiana. To this reviewer's knowledge it is more than anyone else has ever done for an area of similar size.

It is unfortunate that such an excellent work necessarily had to be based on data compiled on the conventional static monthly basis. Otherwise, seasonal contrasts, wet periods, drouths and other periodic events might have been better emphasized and compared. For example, it is difficult to compare percipitation phenomena of 28-day Februaries with 31-day Marches. Wettest 30 days, or driest 30 days, on the basis of long records or for individual years, are seldom, if ever, limited to the period between the first and last day of a given month.

What Deam has done for Indiana flora, Visher has matched in his treatment of the climate of Indiana. To investigators in fields where climatology is an essential adjunct, this work will prove valuable reference—not only for its information, but for its presentation, selection and analysis of date.—*John N. Wolfe.*

The Climate of Indiana, by Stephen S. Visher. 511 pages, 492 figures and 81 tables. Indiana University, Bloomington, Indiana, 1945. $4.00.

Infrared and Raman Spectra

Infrared and Raman Spectra of Polatomic Molecules is the third in a series of books on atomic and molecular spectra by Professor Herzberg. The first concerns itself with atomic spectra alone, while the second on *Diatomic Molecules* is really Volume 1 of the series on molecular spectra. The present volume on Polyatomic Molecules deals with Infrared and Raman Spectra from the points of view of both theoretical treatment of molecular models and the interpretation of experimental data on these models. This book is indispensable to both the beginner and to the man doing independent research in the field of molecular structure. Professor Herzberg has combined an elementary treatment of the problem of the vibration and rotation of molecules with a more advanced point of view. The text material is illustrated with many cases taken from the literature on the subject. Throughout the book are a number of fine print sections which may interest only the specialist, and which may be omitted by the beginner without the loss of continuity. The principal value of the book to the specialist is that it contains at least a thumb nail treatment of most of the molecular types known and extensive tables of both experimental and calculated data. The tables and bibliography are very well cross-referenced, so that where the treatment is not complete the original papers may easily be found. Professor Herzberg has recalculated many constants to fit a more uniform terminology, similar to that used in his *Diatomic Molecules*. The Introduction contains a treatment of Group Theory which should be found useful to many who have no previous knowledge of the subject.

Chapter I gives a general introduction to the subject of Linear, Symmetrical Rotator, Spherical Rotator, and Asymmetrical Rotator molecules in which energy levels, formation of spectra and intensities, are discussed.

In Chapter II, Professor Herzberg deals with the general problem of vibrating systems of particles. Considerable space is devoted to symmetry types of the normal vibrations in a great many molecules. Applications to several specific models, notably, linear and non-linear XY_2, and pyramidal XY_3. The subject of anharmonicities, and isotope effect are also treated.

Chapter III considers the Vibrational Infrared and Haman Spectra from both a Classical and Quantum Mechanical point of view. Following this are specific applications to a great many well-known molecules—CO_2, CS_2, H_2O, C_2H_2, etc.

The problem of the interaction between rotation and vibration is treated in Chapter IV. Combination relations which lead to the calculation of some of the molecular constants are developed. Various models are considered separately with discussions of their band appearance.

Chapter V contains applications of much of the foregoing material to the calculation of thermodynamic quantities.

Infrared and Raman Spectra of Polyatomic Molecules, by Gerhard Herzberg. xiii+632 pp. Fig. 174. 1945. D. Van Nostrand Company, Inc., New York. $9.50.—*Alvin H. Neilsen.*

INDEX TO VOLUME XLV

Agrilus, new species, 80.
Alconeura socorroana Knull n. sp., 103.
Algae and pollens, similarity, 16.
Algologist and water sanitation, 97.
Aves, heart arteries, 19, 167.

Ball, Max W., 29.
Bangham, Ralph V., 82.
Blank, Fritz, 13.
Brown, Harley P., 247.
Brown, J. B., 47.
Bryozoa, Fresh-water, 55.

Callinella aphiodontis n. sp., 111.
Catalogue of Ohio Vascular Plants, 162.
Cisco, copepod parasite of, 82.
Cloanthanus, new species, 22.
Closterium eriense Taft n. sp., 186.
Coleus, multiple alleles in, 170.
Constitution and By-Laws, Ohio Journal of Science, 213.
Copepod parasite of the Cisco, 82.
Cosmarium eriense Taft n. sp., 194.
Cosmarium franzstonii Taft n. sp., 194.
Cosmarium impressulum var. *suborthogona* Taft new comb., 195.
Cosmarium nitidulum var. *pseudovalidum* Taft n. var., 196.
Cosmarium reniforme var. *seminudum* Taft n. var., 199.
Cosmarium subractborskii Taft n. sp., 199.
Cosmarium subnudiceps var. *granulatum* Taft n. var., 199.
Cosmarium viride var. *compressum* Taft n. var., 200.

DeLong, Dwight M., 22.
Desmids, key to genera, 184.

Electronic microscope, use of, 247.
Epiphytology of wheat mosaic, 85.
Erythroneura corylorubra Knull n. sp., 108.
Erythroneura geronimoi Knull n. sp., 108.
Erythroneura kennedyi Knull n. sp., 109.
Erythroneura ohioensis Knull n. sp., 108.
Erythroneura paraesculi Knull n. sp., 106.
Erythroneura stupkaorum Knull n. sp., 104.
Erythroneura trautmanae Knull n. sp., 104.
Erythroneura vagabunda Knull n. sp., 109.
Euastrum ohioense Taft n. sp., 190.

Flagellae, structure of, 247.
Fossil plants, locations in Ohio, 129.
Fox, Rolland David, obituary, 211.
Fueling a global war, 29.

Gaviiformes, heart arteries, 167.
Genetics, human, 13.
Geology, Ohio, 173.
Gliotoxin, 45.
Glenny, Fred H., 19, 167.

Haemopis marmoratis Say, 233.
Herrick, J. Arthur, 45.
Hunter, Margaret Oleson, 47.
Hyalinella punctata, 55.

Inheritance, human, 13.

Johnson, Folke, 85, 125.
Jones, Clyde H., 162.

Kay, Maire Weir, 111.
Knouff, R. A., 47.
Knull, J. N., 80.
Knull, Dorothy J., 103.

Lake Erie, map of, 181.
Latrodectus mactans, 28.
Leafhoppers, new species, 103.
Leech, biology of, 233.
Lepidoptera in Ohio, 18.
Ling Cod parasite, 111.
Lipids, tissue, 47.

Marmor tritici H., 125.
Membership List, Ohio Academy of Science, 223.
Mendenhall, Eugene Warren, obituary, 212.
Micro determination of lipids, 47.
Microtechnic, leech nervous system, 233.
Miller, John A., 233.
Mosaic, wheat, 85, 125.
Multiple Alleles in Coleus, 170.

Nervous system of leech, 233.

Ohio Academy of Science, Annual Report, 206.
Ohio Academy of Science, Constitution and By-Laws, 213.
Ohio Academy of Science, Membership List, 223.
Ohio Lepidoptera, 18.
Ohio Vascular Plants, 162.

Passeriformes—Paridae, 19.
Pennsylvanian Rock Section, 173.
Permian Rock Section, 173.
Peters, John R., obituary, 212
Phytomorula regularis, 16.
Protozoan flagellae, 247.

Rife, David C., 170.
Rogick, Mary Dora, 55.
Ruggy, George H., 115.

Sella Turcica inheritance, 13.
Semans, Frank M., 28.
Snyder, L. H., 13.
Spider, Black Widow, 28.
Staurastrum biarcuus Taft n. sp., 202.

Staurastrum bicoronatium var. *tridentatum* Taft n. var., 202.
Staurastrum brevispinum var. *canadense* Taft n. var., 202.
Staurastrum peleii Taft n. sp., 203.
Staurastrum polytrichum var. *arnatum* Taft n. var., 203.
Stehr, Wm. C., 18.
Stout, Wilbur, 129.
Sulfonamide chemotherapy, 115.

Taft, Clarence E., 16, 97, 180.
Tidd, Wilbur M., 82.
Trematoda, 111.
Trichophyton gypseum, 45.
Typhlocyba foliosa Knull n. sp., 104.
Typhlocyba sciotoensis Knull n. sp., 103.

Vascular Plants of Ohio, 162.

War, Fueling a global, 29.
Water sanitation and the algologist, 97.
Weather Bureau Data, 1.
Wheat mosaic, 85, 125.
White, George W., 173.
Wolfe, John N., 1.